

Effects of Resveratrol Supplementation on Oxidative Damage and Lipid Peroxidation Induced by Strenuous Exercise in Rats

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Abstract

The purpose of the present study was to investigate the effects of resveratrol supplementation on oxidative damage and lipid peroxidation induced by strenuous exercise in rats. The rats were randomly divided into five groups: a sedentary control group, an exercise control group, and three treatment exercise groups administered increasing doses of resveratrol (25, 50, and 100 mg/kg body weight). Resveratrol was administered by oral gavage once daily for four weeks. At the end of the four-week period, the rats performed a strenuous exercise on the treadmill, and the levels of lactate dehydrogenase (LDH), creatine kinase (CK), malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured. The results showed that resveratrol supplementation had protective effects against strenuous exercise-induced oxidative damage and lipid peroxidation by lowering the levels of LDH, CK, MDA, 4-HNE, and 8-OHdG in the serum or muscle of rats. These beneficial effects are probably owing to the inherent antioxidant activities of resveratrol.

Key Words: Resveratrol, Oxidative damage, Lipid peroxidation, Strenuous exercise, Rats

INTRODUCTION

Exercise is known to induce numerous physiological changes in the vital organ systems of the body. The most important exercise-induced change is the enhanced respiration and utilization of oxygen by the body (Kumar and Naidu, 2002). Most of the consumed oxygen is utilized in the mitochondria for substrate metabolism and adenosine 5'-triphosphate (ATP) production, and is eventually reduced to water. However, a small fraction of oxygen (2-5%) may be univalently converted to several intermediates including the superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$), which subsequently leak out of the electron transport chain. $O_2^{\cdot-}$ and $\cdot OH$ are free radicals by definition because they contain an unpaired electron in their atomic structure, whereas H_2O_2 is not. Collectively, they are classified as reactive oxygen species (Ji, 1995). There is growing evidence that strenuous exercise increases the whole body and tissue oxygen consumption by up to 20-fold. As a result, accumulated excessive reactive oxygen species (ROS) has been shown to induce damage to all cellular macromolecules such as lipids, proteins, and DNA, which can induce adverse effects on health and well-being (Aguiló *et al.*, 2005; Misra *et al.*, 2009).

Many studies have indicated that strenuous exercise-induced oxidative damage may be prevented by optimizing nutrition, particularly by increasing the dietary content of nutritional antioxidants. Supplementation with certain antioxidant nutrients is a practical for a fast recovery from fatigue and prevent exercise-induced oxidative damage (Belviranl *et al.*, 2012).

The polyphenolic compound resveratrol (3,5,4'-trihydroxy-trans-stilbene, structure shown in Fig. 1) is a naturally occurring phytochemical that can be found in approximately 72 plant species including food products such as grapes, peanuts, and various herbs (Joe *et al.*, 2002). In plants, resveratrol functions as a phytoalexin that protects against fungal infections (Gehm *et al.*, 1997). Resveratrol has been reported to have a wide range of biological and pharmacological properties including antioxidant, anti-cancer, anti-coagulant, anti-inflammatory,

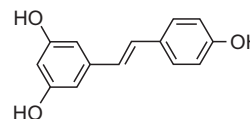


Fig. 1. Structure of resveratrol.

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anti-aging, hypoglycemic, and hypolipidemic (Xie *et al.*, 2013; Nosál *et al.*, 2014; Singh *et al.*, 2015). This compound also inhibits platelet aggregation and exhibits antiestrogenic activity (Stivala *et al.*, 2001). To date, no further information has been reported on the effect of resveratrol on exercise-induced oxidative damage. Therefore, this study was conducted to investigate whether supplementation with resveratrol has any protective effects against strenuous exercise-induced oxidative damage and lipid peroxidation in rats.

MATERIALS AND METHODS

Materials and reagents

Resveratrol (purity >99%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and stored at 2-4°C. The commercial diagnostic kit for the determination of lactate dehydrogenase (LDH) was purchased from Biosino Bio-Technology and Science Inc. (Beijing, China). The commercial diagnostic kits for the determination of creatine kinase (CK) and malondialdehyde (MDA) were purchased from the Jiancheng Biologic Project Co. (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kits for 4-hydroxy-2-nonenal (4-HNE) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were purchased from the Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China) and the Control of Aging, Nikken SEIL Co. Ltd. (Shizuoka, Japan), respectively. The other chemicals used were of analytical grade and acquired from commercial sources.

Animals and breeding conditions

Male Wistar rats weighing between 200-220 g were purchased from the Heilongjiang biological supplier (Harbin, China). The animals were maintained at a temperature of 25 ± 10°C and relative humidity of 45-55% under a 12-h light:12-h dark cycle. The rats were acclimatized for one week prior to the experiments, during which they were fed with a rodent pellet diet and water was provided ad libitum, under strict hygienic conditions.

Exercise protocol

All the experimental protocols described in this study followed the Chinese Guidelines for Animal Experiments (MST-PRC Directive of 1988, No. 88-2) and were approved by the Animal Ethics Committee of the Harbin Normal University (Harbin, China, approval number: HNU 130124). The rats were randomly divided into five groups of eight rats each, which included a sedentary control (SC) group, an exercise control group (EC), and three resveratrol-treated exercise groups (RT-25, RT-50, and RT-100). The resveratrol-treated exercise groups were administered increasing doses of resveratrol (25, 50, and 100 mg/kg body weight) by oral gavage once daily for four weeks. The QC and EC groups were administered saline instead of resveratrol at the same volume. The doses of resveratrol and four-week treatment period selected for this study were based on the results of preliminary experiments.

During the fourth week, the rats were subjected to exercise on a rodent treadmill (Model ZH-PT, Anhui Zhenghua Biological Equipment Co. Ltd., Huaibei, China). The treadmill was equipped with an electric shock device on the rear barrier to motivate the animal to exercise. The speed and duration of

each exercise session were constant at 15 m/min and 15 min for a week, respectively to accustom the rats to running. At the end of the four-week period, the rats in the EC, RT-25, RT-50, and RT-100 groups performed a strenuous exercise on the treadmill at a final speed of 30 m/min, 10% gradient, and approximately 70-75% maximal oxygen consumption (VO₂max). Exhaustion was determined as the point when the rat was unable to right itself when placed on its back (Shan *et al.*, 2011).

Sample preparation

After the completion of the exhaustive exercise, the rats were anaesthetized using diethyl ether, euthanized, and then blood samples were collected via abdominal aorta puncture and the serum was isolated. The serum samples were then stored at -80°C until required for the LDH, CK, 4-HNE, MDA, and 8-OHdG analyses. The gastrocnemius muscle was also removed, washed with physiological saline, and frozen in liquid nitrogen for storage at -80°C until required for the 4-HNE, MDA, and 8-OHdG analyses. All the measurements using the commercial kits were carried out in accordance with the instructions from manufacturers.

Statistical methods

The statistical analysis was performed using the statistical package for the social sciences (SPSS) version 11.0 SPSS Inc., Chicago, IL, USA). The data are expressed as mean ± standard deviation (SD) and were analyzed using the one-way analysis of variance (ANOVA) followed by Turkey's post hoc test. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Effects of resveratrol on exhaustive exercise times of rats

The laboratory rat is a commonly used animal model for investigating the effects of exhaustive exercise on biochemical parameter for extrapolation to humans. In this study, a rat model was used to investigate the exhaustive exercise-induced oxidative damage and lipid peroxidation. As shown in Fig. 2, the exhaustive exercise times of the RT-25, RT-50, and RT-100 groups were significantly longer than that of the

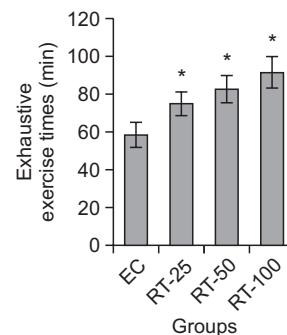


Fig. 2. Effects of resveratrol on exhaustive exercise times of rats. EC, exercise control rats treated with saline for four weeks; RT-25, RT-50, and RT-100, exercise rats treated with resveratrol (25, 50, and 100 mg/kg body weight, respectively) for four weeks. Data are expressed as mean ± SD of eight rats in each group. * $p < 0.05$ compared with EC group.

EC group ($p < 0.05$). The results indicate that resveratrol might possess an anti-fatigue effect.

Effects of resveratrol on serum lactate dehydrogenase and creatine kinase levels in rats

The exhaustive exercise-induced oxidative muscle damage in rats was estimated by measuring the serum levels of LDH and CK. As shown in Fig. 3, the serum LDH levels of the SC, RT-25, RT-50, and RT-100 groups, as well as the serum CK levels of the SC, RT-50, and RT-100 groups, were significantly lower than those of the EC group were ($p < 0.05$). The serum LDH levels of the EC, RT-25, and RT-50 groups as well as the serum CK levels of the EC, RT-25, RT-50, and RT-100 groups were significantly higher than those of the SC group were ($p < 0.05$).

Effects of resveratrol on serum and muscle malondialdehyde and 4-hydroxy-2-nonenal levels in rats

The exhaustive exercise-induced lipid peroxidation in rats was estimated by measuring the serum and muscle levels of MDA and 4-HNE. As shown in Fig. 4A, the serum MDA lev-

els of the SC, RT-25, RT-50, and RT-100 groups, as well as the muscle MDA levels of the SC, RT-50, and RT-100 groups, were significantly lower than those of the EC group were ($p < 0.05$). The serum MDA levels of the EC, RT-25, and RT-50 groups as well as the muscle MDA levels of the EC, RT-25, RT-50, and RT-100 groups were significantly higher than those of the SC group were ($p < 0.05$). As shown in Fig. 4B, the serum and muscle 4-HNE levels of the SC, RT-25, RT-50, and RT-100 groups were significantly lower than those of the EC group were ($p < 0.05$). The serum 4-HNE levels of the EC, RT-25, and RT-50 groups as well as the muscle 4-HNE levels of the EC, RT-25, RT-50, and RT-100 groups were significantly higher than those of the SC group were ($p < 0.05$).

Effects of resveratrol on serum and muscle 8-hydroxy-2'-deoxyguanosine levels of rats

The exhaustive exercise-induced oxidative DNA damage in rats was estimated by measuring the serum and muscle 8-OHdG levels. As shown in Fig. 5, the serum and muscle 8-OHdG levels of the SC, RT-25, RT-50, and RT-100 groups were significantly lower than those of the EC group were ($p < 0.05$). The serum and muscle 8-OHdG levels of the EC, RT-25, RT-50, and RT-100 groups were significantly higher than those of the SC group were ($p < 0.05$).

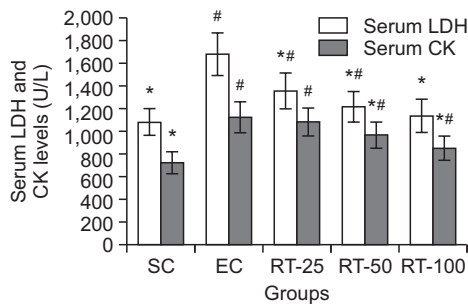


Fig. 3. Effects of resveratrol on serum lactate dehydrogenase and creatine kinase levels in rats. SC, sedentary control rats treated with saline; EC, exercise control rats treated with saline; RT-25, RT-50, and RT-100, exercise rats treated with resveratrol (25, 50, and 100 mg/kg body weight). All groups were treated for four weeks. Data are expressed as mean \pm SD of eight rats in each group; * $p < 0.05$ compared with EC group, # $p < 0.05$ compared with SC group.

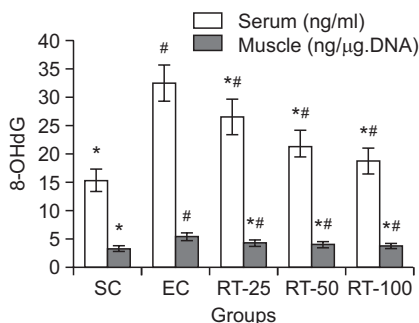


Fig. 5. Effects of resveratrol on serum and muscle 8-hydroxy-2'-deoxyguanosine levels of rats. SC, sedentary control rats treated with saline; EC, exercise rats treated with saline; RT-25, RT-50, and RT-100, exercise rats treated with resveratrol (25, 50, and 100 mg/kg body weight, respectively). All groups were treated for four weeks. Data are expressed as mean \pm SD; * $p < 0.05$ compared with EC group, # $p < 0.05$ compared with SC group.

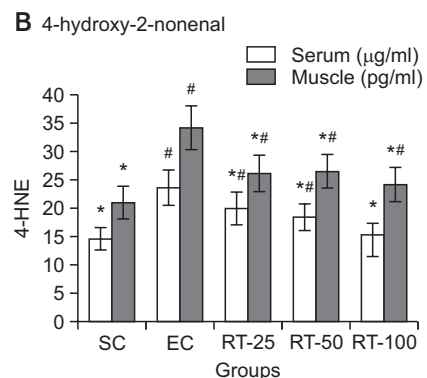
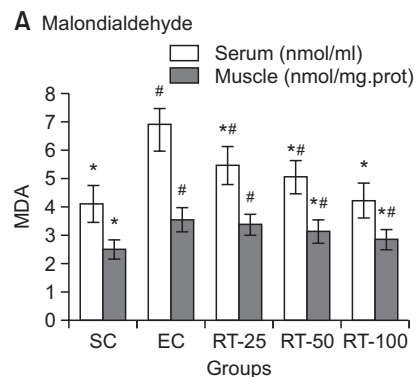


Fig. 4. Effects of resveratrol on serum and muscle malondialdehyde and 4-hydroxy-2-nonenal levels in rats. SC, sedentary control rats treated with saline; EC, exercise control rats treated with saline; RT-25, RT-50, and RT-100, exercise rats treated with resveratrol (25, 50, and 100 mg/kg body weight). All groups were treated for four weeks. Data are expressed as mean \pm SD of eight rats in each group; * $p < 0.05$ compared with EC group, # $p < 0.05$ compared with SC group.

DISCUSSION

This study was designed to investigate the effects of resveratrol supplementation on oxidative damage and lipid peroxidation induced by strenuous exercise in rats. The major findings of the effects of resveratrol supplementation in rats were as follows: 1) a significant increase in exhaustive exercise times; 2) a significant decrease in serum and muscle LDH and CK levels after exhaustive exercise; and 3) a significant decrease in serum and muscle MDA, 4-HNE, and 8-OHdG levels after exhaustive exercise.

Oxidative muscle damage has been widely reported following strenuous exercise (Ceci *et al.*, 2015). LDH, which is an enzyme expressed mainly in the sarcoplasm of skeletal muscle, is responsible for the removal of lactate produced after fast anaerobic consumption of glucose during muscle contraction (Lu *et al.*, 2006). Under normal condition, the activity of serum LDH reflects the degree of lactate metabolism by the body and the extent of basal cell damage. High serum LDH activity may signify leakage of the enzyme from the muscle cells that have been damaged by per-oxidative injury, a crash, or symptoms of other diseases (Lu *et al.*, 2006). It has been suggested that this damage leads to temporary loss of the capacity of muscle to produce adequate force during exercise. In addition, this damage is implicated in the increase in muscle soreness experienced post exercise (You *et al.*, 2011). The normal function of CK in cells is to add a phosphate group to creatine, thereby converting it into the high-energy molecule phosphocreatine, which is metabolized by the cells as a quick source of energy. However, the function of CK has a greater relevance to what occurs in damaged muscles. During the process of muscle degeneration, the muscle cells lyse, and their contents are released into the bloodstream. Because most of the CK in the body normally exists in the muscle, an increase in the blood levels of CK indicates that muscle damage has occurred or is being initiated (Qi *et al.*, 2014). There is increasing evidence that the high levels of serum CK and LDH observed after the cycling stage may result from a modest disruption of the muscle cell membrane, which consequent allows the leakage of proteins (Tauler *et al.*, 2006; Ostman *et al.*, 2012). In this study, the results showed that the levels of serum CK and LDH of the rats in the RT-50 and RT-100 groups were significantly lower than those of the control group were. The results indicate that resveratrol decreases muscle damage following exhaustive exercise.

Lipid peroxidation appears to be an important mechanism underlying exercise-induced muscle damage (Sacheck and Blumberg, 2001). At the molecular level, the production of ROS such as the $O_2^{\cdot-}$ is an essential event in the formation of the $\cdot OH$, which can then extract a hydrogen atom from the cellular membrane lipids. This action results in the autocatalytic production of lipid radicals and the peroxidative degradation of membrane lipids (Sampey *et al.*, 2003). There is increasing evidence that lipid peroxidation may be especially harmful. The various lipid-derived products resulting from lipid peroxidation such as MDA, 4-HNE, and acrolein have demonstrated multiple biological or toxicological effects. The 4-HNE and MDA are the most abundant aldehydes produced while acrolein is the most reactive (Perluigi *et al.*, 2012). These molecules have longer half-lives than ROS and the potential to diffuse from their site of origin to distant intracellular and extracellular targets, thereby amplifying the effects of oxidative

stress (Browning and Horton, 2004). In this study, the results showed that the serum and muscle levels of 4-HNE and MDA of the rats in the RT-50 and RT-100 groups were significantly lower than those of the control group were. The results indicate that resveratrol decreases lipid peroxidation following strenuous exercise.

Strenuous physical exercise with dramatically increased oxygen uptake is associated with the generation of ROS. These ROS cause extensive DNA damage including single-strand breaks and the formation of modified bases (Rahimi, 2011). Over the past decades, many studies have accumulated evidence that strenuous exercise induces oxidative damage to DNA and increases the formation of methylated bases (Morillas-Ruiz *et al.*, 2005). Oxidative damage to DNA is generally measured by the levels of 8-OHdG, and its excretion reflects the integrated rate of oxidative DNA damage and its repair in the whole body (Sato *et al.*, 2010). In this study, the results showed that the serum and muscle 8-OHdG levels of the rats in the RT-25, RT-50, and RT-100 groups were significantly lower than those of the control group were. The results indicate that resveratrol decreases DNA damage and increases its repair during strenuous exercise.

CONCLUSIONS

The present study clearly demonstrates that resveratrol supplementation has protective effects against strenuous exercise-induced oxidative damage and lipid peroxidation in rats. Furthermore, this effect is mediated by the lowering of the serum and muscle levels of LDH, CK, MDA, 4-HNE, and 8-OHdG. These beneficial effects are probably attributable to the inherent antioxidant activities of resveratrol, and require further investigation to determine the mode of its kinetics. This information would contribute to the development of resveratrol for possible medicinal applications in exercise-induced oxidative damage.

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