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Full Length Research Paper

Beneficial effect of curcumin isolated from *Curcuma longa* on exercise-induced hepatocyte apoptosis of rat

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The aim of this study was to determine the effect of curcumin isolated from *curcuma longa* on exercise-induced hepatocyte apoptosis. 24 male Wistar rats were used for the study. The experiment was a 14-day exhaustive exercise. We determined the protein expression of bax and bcl-2 in the hepatic tissues. The results demonstrated that bax and bcl-2 co-regulated the exercise-induced hepatocyte apoptosis. Bax promotes the apoptosis as up-regulated genes, while bcl-2 inhibits apoptosis as down-regulated genes. Curcumin can inhibit exercise-induced hepatocyte apoptosis. The experimental results provided theoretical support for curcumin in the field of sports nutrition.

Key words: Curcumin, exercise, apoptosis.

INTRODUCTION

Multicellular organisms eliminate redundant, damaged, or infected cells by a stereotypic program of cell suicide termed apoptosis (Kerr et al., 1972; Adams and Cory, 1998; Kujoth et al., 2005). Thompson reported that apoptosis is a form of cell death that constitutes part of a common mechanism in cell replacement, tissue remodeling and removal of damaged cells (Thompson, 1995). Inappropriate or excessive apoptosis, however, has been implicated in many diseases including cancer, autoimmune diseases, neurodegenerative disorders, acquired immunodeficiency syndrome (AIDS) and stroke (Thompson, 1995; Sim et al., 2004). Recently, 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 exercise (Phaneuf and Leeuwenburgh, 2001; Mooren et al., 2004; Wang and Huang, 2005; Siu et al., 2004; Camara et al., 2007). Apoptosis is orchestrated by the sequential activation of caspases, a family of cysteine proteases with specificity for aspartic acid residues. Two pathways may mediate apoptosis:

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(1) The death receptors pathway (tumour necrosis factor, Fas ligand or TRAIL receptors) that activates caspase 8 and 10.
(2) The mitochondrial pathway that activates caspase 9. Both pathways lead to effector caspases (3, 6 and 7) (Igney and Krammer, 2002). Bcl-2 is antiapoptotic for the mitochondria pathway, whereas bax is a proapoptotic factor activated by p53 (Guo et al., 2002; Zhu et al., 2006; Gustafsson and Gottlieb, 2007).

Turmeric is a spice which is obtained from rhizomes of plant *Curcuma longa*, a member of the family Zingaberaceae. Main components of turmeric are named curcumin (diferuloyl methane), a low molecular weight polyphenol (Wu, 2003) (Figure 1). Curcumin is known for its antioxidant, anti-inflammatory, anti-fatigue, anti-parasitic, antiallergic, anti-microbial, anti-mutagenic and anticancer properties (Reddy and Lokesh, 1992; Srimal, 1997; Li and Liu, 2005; Xiong and Chi, 2005; Choi et al., 2006; De et al., 2009; Kaewnopparat et al., 2009). Therefore, it is regarded as a high potential to develop into modern drug. In our pre- research it had been further confirmed that curcumin has antioxidant activity *in vitro* and it can enlarge strength of mice. The purpose of the present research was to determine the effect of curcumin on exercise-induced apoptosis in trained rats. We

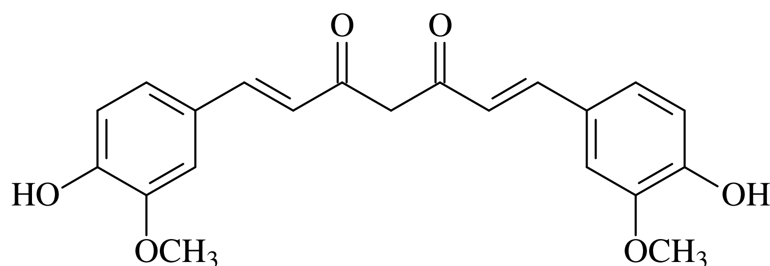

Figure 1. Chemical structures of curcumin.

Table 1. Experimental groups.

Group Ⅰ (NC)	normal control rats were gavaged with 0.5% CMC daily for 14 days
Group Ⅱ (EC)	exercises control rats were gavaged with 0.5% CMC daily for 14 days
Group Ⅲ (CT)	curcumin treated rats were gavaged with75 mg/kg curcumin daily for 14 days

evaluated the expression of bcl-2 and bax in hepatic tissues after exhaustive exercise in order to clarify its mechanisms.

MATERIALS AND METHODS

Plant material

The dried turmeric was purchased from the Tianjin Herbal Medicine Market, China and the plant was identified by Prof. Li, Tianjin Agricultural University China. The voucher specimens of this plant were deposited at the Herbarium, Tianjin Agricultural University.

Isolation of pure curcumin

Isolation of pure curcumin was as described previously (Rao et al., 1970). The dried turmeric was crushed in an electrical grinder and then powdered. 100 g of this powder was extracted in a soxhlet type extractor with absolute ethanol for 8 h. Ethanol was removed from the extract at 50℃ in a flash evaporator, superior kerosene was added to the residue, thoroughly shaken and the kerosene decanted off. Treatment with kerosene was repeated thrice, which removed most of the resin. To the residue, petroleum ether (40 to 60℃) was added, thoroughly mixed and the solvent decanted. Crude curcumin so obtained was further purified by recrystallization from hot absolute ethanol (melting point 174℃).

Animals and exercise

24 male Wistar rats (weight: 220 - 250 g) were used for this study. They were housed under controlled environmental conditions of temperature (22 ± 2℃) and a 12 h light/dark cycle and maintained on (unless otherwise stated) standard food pellets and tap water *ad libitum*. All animal handling procedures were performed in strict accordance with the China legislation of the use and care of laboratory animals, with the guidelines established by Institute for Experimental Animals of Tianjin Agricultural University and were approved by the ad hoc ethical committee of the University for Animal Experiments.

Rats were adapted to conditions for 1 week before the experiment began. The animals were randomly divided into the following three groups, each group consisting of eight rats (Table 1).

The exhaustive experimental rats underwent a 14-day exhaustive exercise program. The run to exhaustion consisted of a single treadmill challenge at 35 m/min and a slope of 9%. Rats were made to run at this speed and slope until they were unable to respond to continuous prodding with a soft brush (Ni et al., 2008). The mean time to exhaustion was 110 min (range: 80 - 125 min). The rats were trained for 6 days per week, that is, Monday, Tuesday, Thursday, Friday and Saturday. Each time the training began from 9 a.m.

Tissue preparation

At the end of exhaustive experiment, all rats were anaesthetized with sodium pentobarbital (40 mg/kg body weight) and were killed by decapitation. The hepatic tissues were extracted from rats and the obtained tissues were 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 6 um thickness.

Immunohistochemical assays

Immunohistochemical staining was adopted to test the protein expression of bax and bcl-2 in the hepatic tissues of the rats. After bax and bcl-2 protein expression of hepatic tissues was observed under light microscope, the comprehensive judgment was carried out based on the percentage of positive cells. The presence of brown punctate or granular substances in the cells was an indication of positive staining.

Statistical analysis

The data were analyzed by using SPSS (version 10.0). All values were expressed as mean ± SD. When comparing groups, statistical

Table 2. The comparison of bax, bcl-2 and bax/bcl -2 ratio among three groups (n = 8).

Group	Bax	Bcl-2	Bax/bcl-2
I (NC)	11.36 ± 3.48	18.47 ± 3.61∆	0.62∆
II (EC)	21.63 ± 4.67*	12.53 ± 4.19*	1.73*
III (CT)	15.34 ± 4.11 *∆	16.82 ± 4.57∆	0.91*∆

Note: *p < 0.05, as compared with normal control group; ∆ p < 0.05, as compared with exhausting exercises control.

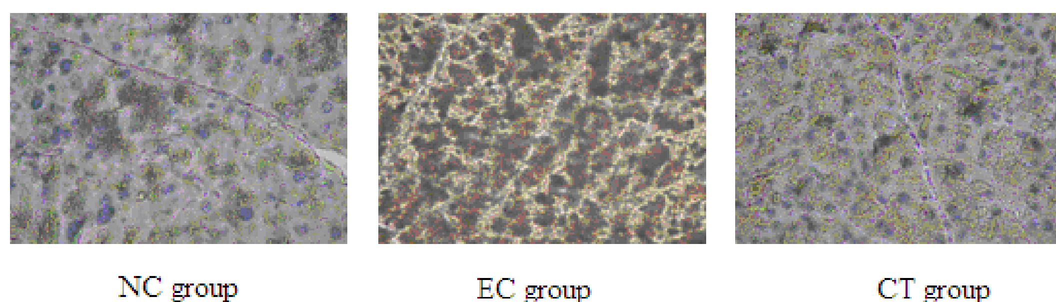

Figure 2. Change in bax protein expression in hepatic tissues of rats.

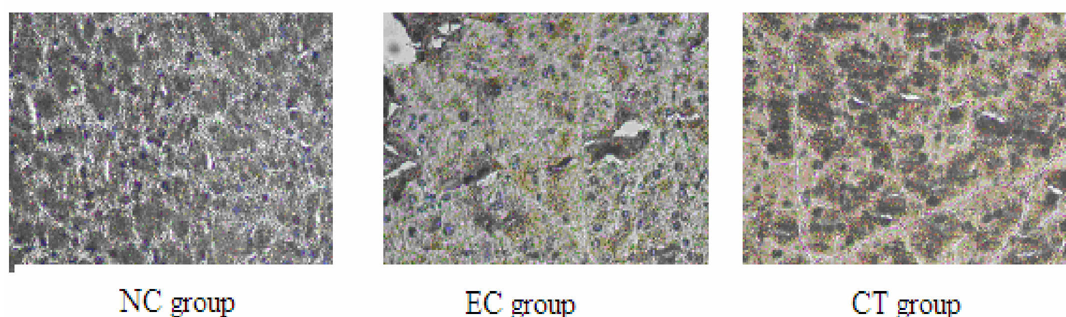

Figure 3. Change in bcl-2 protein expression in hepatic tissues of rats.

significance was determined by using one-way ANOVA. If a statistically significant difference was observed, Student Newman–Keul post hoc test was used. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 2, Figure 2 and Figure 3, compared EC group and CT group with NC group, the protein expression of bax had significant statistic difference ($p < 0.05$) and compared CT group with EC group, the protein expression of bax had also significant statistic difference ($p < 0.05$). Compared EC group with NC group, the protein expression of bcl-2 had significant statistic difference ($p < 0.05$); compared NC group and CT group with EC group, the protein expression of bcl-2 had also significant statistic difference ($p < 0.05$). Compared EC group and CT group with NC group, the bax/bcl -2 ratio had significant statistical difference ($p < 0.05$); compared NC group and CT group with EC group, the bax/bcl -2 ratio had also significant statistic difference ($p < 0.05$).

Inhibitors of apoptosis, such as bcl-2 protein, and inducers, such as bax, have been identified (Korsmeyer, 1992; Itoh et al., 1993; Hockenbery et al., 1993; Oltvai et al., 1993; Miyashita et al., 1994; Hanada et al., 1995). The bcl-2 protein (molecular weight of ≈ 25 kD) is encoded by a gene involved in the 14:18 chromosomal translocation (Cleary and Sklar, 1985; Tsujimoto et al., 1985; Pawlowski and Kraft, 2000; Campbell et al., 2007). The bcl-2 protein is a cytosolic protein with a lipid anchoring domain that

can target it to the mitochondria or the nucleus (Tsujimoto et al., 1985). It plays a role in the inhibition of apoptosis (Tsujimoto et al., 1985; Hanada et al., 1995; Delchev et al., 2006). Bax, a member of the bcl-2 family, is encoded by six exons (Delchev et al., 2006). It homodimerizes and forms heterodimers with bcl-2 *in vitro*. Over expressed bax accelerates apoptotic death. Bax/bcl-2 ratio determines survival or death after an apoptotic stimulus (Miyashita et al., 1994; Yang et al., 1995; Pavlović et al., 2007). Our study showed that exhausting exercises could make bax protein expression in hepatic tissues and bax/bcl-2 ratio dramatically increases, bcl-2 protein expression obviously decreases. The results indicated that bax and bcl-2 co-regulated the exercise-induced hepatocyte apoptosis. 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 hepatocyte apoptosis. While after rats were fed curcumin by gavages, the bax protein expression and bax/bcl-2 ratio obviously decreased, bcl-2 protein expression obviously increased. The results indicated that curcumin can inhibit exercise-induced hepatocyte apoptosis of rats.

Conclusion

Apoptosis is a partially reversible process and therefore, therapeutic interventions could be tested to attenuate this process. After rats were fed curcumin by gavages, the bax protein expression and bax/bcl-2 ratio obviously decreased, bcl-2 protein expression obviously increased. Curcumin can inhibit exercise-induced hepatocyte apoptosis. The experimental results provided theoretical support for curcumin in the field of sports nutrition.

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