Protective effect and mechanism of EGCG on acute lower limb ischemia-reperfusion injury in rabbits

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Abstract

AIM: To investigate the potential beneficial effect of epigallocatechin gallate (EGCG) in ischemia/reperfusion (I/R) injury of skeletal muscle. METHODS: Eighteen adult male New Zealand rabbits were allocated into 3 groups：(1) Sham operation group（SO）, (2) I/R with normal saline group (I/R), (3) I/R with EGCG group (EGCG). A rabbit model of skeletal muscle I/R injury was induced by 4-h hindlimb ischaemia and 4-h reperfusion. Normal saline and EGCG (10 mg/kg) were administered in trapper it on eally at 10 min before reperfusion, respectively. Muscle samples were analyzed for detecting the levels of super oxide dismutase (SOD) and malondialdehyde (MDA). Muscle samples were assessed by hematoxylin and eosin (H&E) . RESULTS: Muscle tissues and serum of the I/R group had significantly increased levels of MDA (P < 0.01), and decreased SOD activities compared with the sham group (P<0.05). The activity of SOD in the IR with EGCG group was greatly elevated compared with that in the I/R group (P<0.05). CONCLUSIONS: From the histological and serum biochemical perspective, the treatment with EGCG has efficiently alleviated the injuries in the skeletal muscle ischemia and reperfusion in this experimental model.

Keywords

Ischemia/reperfusion injury, Epigallocatechin gallate, Oxidative stress

1. Introduction

In many vascular and muscle trauma and limb surgery, skeletal muscle ischemia is inevitable, and the recovery of blood supply often leads to more serious reperfusion injury [1]. After the ischemic muscle tissue recovers the oxygenated blood flow, free oxygen free radicals and activated neutrophils follow [2]. Acute inflammatory response is the pathophysiological basis of ischemia-reperfusion injury (IRI) [3-4].

Epigallocatechin gallate (EGCG) is the main active chemical component derived from green tea polyphenols. It has many biological functions, such as anti-oxidation, anti-inflammatory response, anti apoptosis and so on. Previous animal experiments have shown that EGCG can inhibit the expression of inflammatory factors and reduce the ischemia-reperfusion injury of heart, liver, kidney and other organs. However, the effect and exact mechanism of EGCG on skeletal muscle ischemia-reperfusion injury have not been reported. Therefore, this study observed the protective effect of EGCG on lower limb ischemia-reperfusion in rabbits, and further explored the potential mechanism of EGCG on inhibiting the inflammatory process in ischemia-reperfusion injury.

2. Materials and Methods

2.1 Management of experimental animals

All animal experiments were conducted in accordance with the guidelines of the medical ethics committee of Jianghan University. Eighteen male adult New Zealand rabbits (body weight 2.55 ± 0.37 kg) were purchased from Hubei experimental animal research center. They were randomly divided into three groups with 6 rabbits in each group: (1) sham operation group (sham), (2) ischemia-

reperfusion injury control group (I/R + saline), (3) ischemia-reperfusion injury EGCG treatment group (I/R + EGCG). Eat and drink freely. Fasting 12 hours before operation could not help water.

The IRI model of rabbit lower limbs was established by referring to the methods of Crinnion et al. 1.2% Pentobarbital Sodium was injected into the ear vein at the dose of 3 ml/kg, the right femoral artery and vein were exposed at the right femoral triangle, and then the femoral artery and vein were clamped with sterile microvascular clamp. At the same time, the limb was bound along the groin with a tourniquet to block the collateral circulation, resulting in complete ischemia of the lower limbs. In the sham operation group, only the femoral artery and vein were exposed Vein without clipping and cerclage. Ischemia treatment for 4h, reperfusion for 4h, and 10 min before blood flow recovery, the I/R + saline group was given 20ml normal saline, and the I / R + EGCG group was given 10 mg/kg EGCG (dissolved in normal saline, 0.2 μM filtration). EGCG was purchased from Hangzhou pulimedi Biotechnology Co., Ltd..

2.2 Collection of serum and tissue samples

After 4 hours of perfusion, the inferior vena cava blood was collected, centrifuged at 2500 g at 4 ℃ for 15 min, and the serum was separated for testing. Free the gastrocnemius muscle of the right lower limb, take 1 * 1cm and fix it in 4% neutral paraformaldehyde solution, then embed it in paraffin, and the rest will be frozen at －80 ℃ after quick freezing with liquid nitrogen.

2.3 Biochemical detection

The gastrocnemius muscle tissue was homogenized in 3 times the volume of precooled Tris HCl buffer (pH 7.0), centrifuged at 3500 g at 4 ℃ for 30 min, and the supernatant was collected for testing. The contents of malondialdehyde (MDA) and superoxide dismutase (SOD) in serum and muscle homogenate were detected according to the instructions of the kit. MDA (cat: s0101) and SOD (cat: s0131) test kits were purchased from Shanghai biyuntian Biotechnology Co., Ltd..

2.4 Histological examination

The gastrocnemius muscle fixed by neutral paraformaldehyde was embedded in stone wax for 5 years μ M serial sections were stained with hematoxylin eosin. Each section was randomly selected from 10 visual fields under 40x light microscope to observe and evaluate the pathological damage rating. The scoring standard of histological injury refers to that recommended by erkanli et al., that is, loose and dissolved muscle fibers: normal (0), slight (1), moderate (2), severe (3), inflammatory cell infiltration: normal (0), slight (1), moderate (2), severe (3).

2.5 TUNEL detection

TUNEL detection of apoptosis was carried out according to the instructions of the detection kit. The positive staining measurement was analyzed with images Pro Plus 6.0 software and expressed as integrated optical density (IOD). TUNEL test kit (cat: c1091) was purchased from Shanghai biyuntian Biotechnology Co., Ltd.

2.6 Statistical analysis

The histological injury score was expressed as mean ± quartile difference, and the other data were expressed as mean ± standard deviation. All data were analyzed by graphpad prism 6 software. The histological score was tested by kruskale Wallis h test, with a significant level $\alpha = 0.05$； The other data were analyzed by one-way ANOVA with significant level $\alpha = 0.05$。

3. Results

3.1 EGCG reduce the edema of gastrocnemius muscle tissue, dissolution and necrosis of muscle fibers caused by ischemia-reperfusion injury.

The normal gastrocnemius muscle fibers have clear boundaries and uniform morphology, without holes or fractures, while the damaged gastrocnemius muscle fibers have uneven and broken morphology. As shown in Figure 1: compared with the sham operation group, the gastrocnemius muscle fibers in the ischemia-reperfusion group were arranged loosely and irregularly, the muscle

membrane was incomplete and fuzzy, the muscle transverse lines were disordered, significant muscle fiber interstitial edema occurred, and muscle fiber dissolution and necrosis occurred in some areas. Compared with ischemia-reperfusion group, the continuity and integrity of muscle fibers and the degree of interstitial edema in EGCG group were significantly improved.

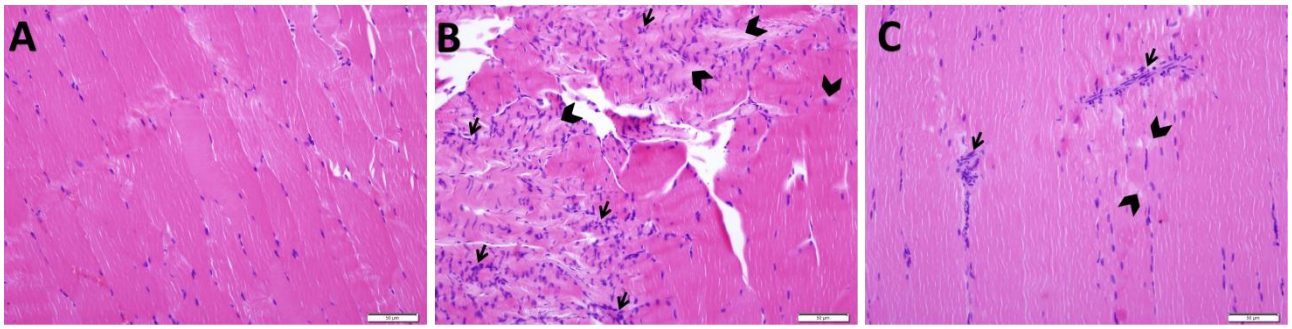

Figure 1: Hematoxylin eosin staining of gastrocnemius muscle in each group. A is the sham operation group; B is ischemia-reperfusion group; C is EGCG group

3.2 EGCG decreased the level of MDA and increased the level of SOD in gastrocnemius muscle during ischemia-reperfusion injurye

As shown in Figure 2, compared with the sham operation group, the level of MDA in rabbit gastrocnemius muscle in the ischemia-reperfusion group increased significantly ($P < 0.01$), and the level of superoxide dismutase decreased significantly ($P < 0.01$). Compared with ischemia-reperfusion group, the level of MDA in rabbit gastrocnemius muscle in EGCG (200 mg / kg) group decreased significantly ($P < 0.01$), and the level of superoxide dismutase increased significantly ($P < 0.01$). Correlation analysis showed that there was a high correlation between the concentration of EGCG and the levels of MDA and superoxide dismutase.

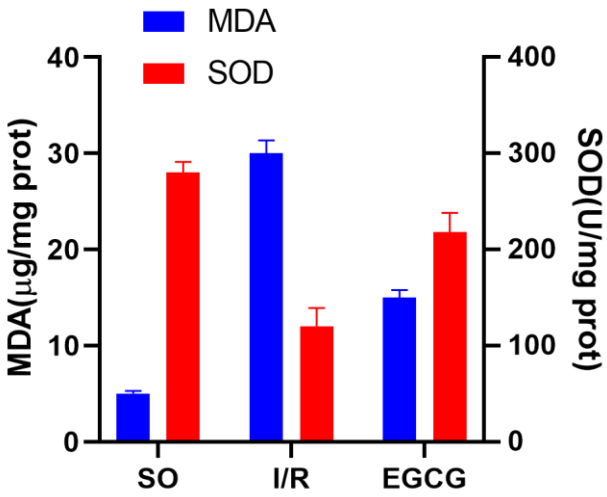

Figure 2: Levels of MDA and SOD in gastrocnemius muscle of each group.

3.3 EGCG inhibits apoptosis of gastrocnemius muscle during ischemia-reperfusion

As shown in Figure 3: compared with the sham operation group, brown particles exist in most nuclei in the ischemia-reperfusion group, that is, most cells have been apoptotic. Compared with ischemia-reperfusion group, EGCG positive cells decreased significantly. After counting the cells by ImageJ software, the apoptosis rate of gastrocnemius muscle cells in each group was calculated and statistically analyzed. Compared with the sham operation group, the apoptosis rate of ischemia-reperfusion group increased significantly ($P < 0.01$); Compared with ischemia-reperfusion group, the apoptosis rate of EGCG group decreased ($P < 0.05$).

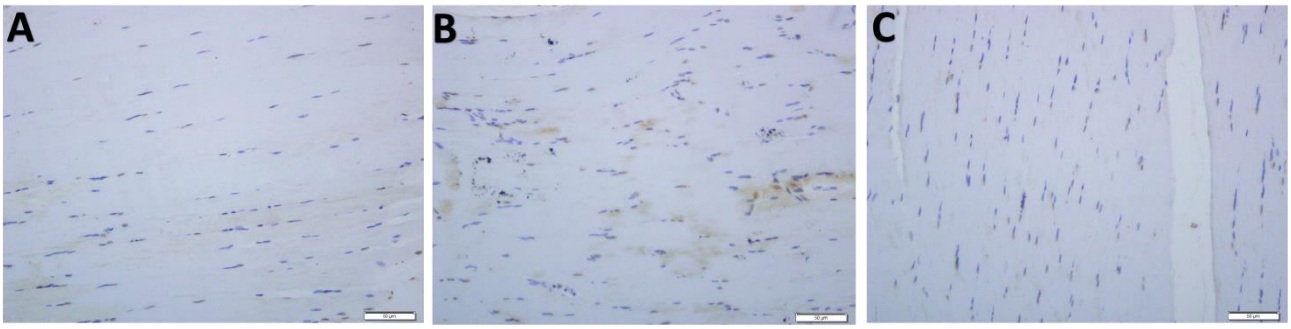

Figure 3: Apoptosis of gastrocnemius muscle in each group (TUNEL staining).

4. Conclusion

This study shows that EGCG can reduce skeletal muscle injury caused by ischemia-reperfusion. In addition, EGCG has a protective effect on oxidative stress and systemic inflammatory response induced by ischemia-reperfusion injury, and may be a promising drug for the treatment of ischemia-reperfusion injury.

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