Int J Clin Exp Med 2014;7(12):5730-5736
www.ijcem.com /ISSN:1940-5901/IJCEM0002648

Original Article

Protective effect of epimedium combined with oligomeric proanthocyanidins on exercise-induced renal ischemia-reperfusion injury of rats

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Received September 20, 2014; Accepted November 25, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: Objective: This paper studied the protective effect and mechanism of epimedium combined with oligomeric proanthocyanidins on exercise-induced renal ischemia-reperfusion injury of rats. Methods: In the experiment, the rats were given exhaustive swimming training and then their blood urea nitrogen (BUN) and other biochemical indexes were measured after they were given gastric perfusion with 6.01 g/kg doze of epimedium and 50 mg/kg doze of oligomeric proanthocyanidins for 56 days. Results: The result indicated that 8 weeks of over training led to ischemia-reperfusion injury of rats. Moreover, their kidney tissues were significantly changed pathologically and renal functions drastically damaged. BUN and serum creatinine increased and EOM group ($P < 0.05$), OPCOM group ($P < 0.05$) and EOPCOM group ($P < 0.01$) were lower than OM group. EOPCOM group was lower than OPCOM group. SOD activity decreased, EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), EOPCOM group ($P < 0.01$) higher than OM group, and EOPCOM group ($P < 0.05$) higher than OPCOM group. The content of MDA increased, EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), EOPCOM group ($P < 0.01$) lower than OM group, and EOPCOM group ($P < 0.05$) lower than OPCOM group. Conclusion: Both epimedium and oligomeric proanthocyanidins can boost SOD activity, clean oxygen radicals, clean and alleviate peroxidation of lipids, which exert protection on exercise-induced renal ischemia-reperfusion. The two combined yield a much better result.

Keywords: Epimedium, oligomeric proanthocyanidins, exercise-induced ischemia-reperfusion

Introduction

With the theoretical development of modern traditional Chinese medicine, pharmacology and biotechnology, more single doses and mixed doses are used in sports practices. Traditional Chinese medicine is increasingly showing its unique advantages because it is multi-targeted in various ways and it hardly contains any prohibited ingredients. Traditional Chinese medicine believes that over-training can damage one's vitality and accordingly exhaust one's strength [1, 2]. Tiredness cause by limit load training damages the spleen in the beginning and the kidney eventually. The kidney is the foundation of the body and it governs bones and generates the marrow. When the body is stable, renal blood flow can maintain stability by self-regulation. But in extreme sports, renal blood flow changes under the influence of nerves and body liquids. The redistribution of blood flow in various organs increases the blood flow in organs in motion, muscles in particular and decreases renal blood flow drastically. The decrease becomes more evident as sports duration and intensity increase. This incomplete lack of blood leads to exercise-induced renal ischemia. After sports stop, renal blood supply leads to reperfusion after ischemia. In this process, the unusual increase of radical generation plays an important role [3-5]. Studies have shown that epimedium can boost the activity of superoxide dismutase; oligomeric proanthocyanidins (OPC), a naturally powerful antioxidant, is capable of removing radicals and fortify the cellular defense system of antioxidation [6, 7]. Compared with vitamins C and E, its power to

erase oxygen radicals is 50 times as strong as Vitamin C and 20 times as strong as Vitamin E, as powerful as SOD. Early studies also reveal that epimedium combined with Vitamin C has a protective effect on exercise-induced renal ischemia reperfusion. So what is the possible result if epimedium is used together with oligomeric proanthocyanidins, which are 20 times as powerful as Vitamin C in terms of their ability to remove oxygen radicals? This paper studies the effect of epimedium combined with oligomeric proanthocyanidins on renal ischemia-reperfusion of rats, and explores the role and mechanism of the two in exercise-induced ischemia reperfusion so as to provide theoretical basis for clinical study.

Materials and methods

Animals

A total of 100 clean male Wistar rats, 42 days old, average weight (197.2 ± 15.1) g, provided by experimental animal department, medical school, Beijing University, license number SCXK (Beijing) 2006-0008. During the whole experimental process, the lab temperature was kept at (22 ± 2)°C, relative humidity 55%~75%, and illumination time changed as nature did. All experimental rats were conventionally bred with basic diet (provided by experimental animal department, medical school, Beijing University) and distilled water. The experimental duration was 63 days and the formal training 56 days.

Experimental medicine

Epimedium (batch number 130125437), made in Jilin, purchased from Beijing Tong Ren Tang, was verified by Gao Zhanyou, a senior engineer with Swiss Tianjin Pharmaceutical Co., Ltd. A fixed amount of traditional Chinese medicine was scaled, boiled, filtered, condensed into 1 g crude drug/mL and put aside for use. Oligomeric proanthocyanidins, OPC (batch number 201301052), provided by Xinjiang-star technology investment Co., Ltd., was made up with distilled water into 10 mg/mL solution.

Major instruments

BS224S electronic analytic balance (Germany Sartorius), optical microscopes (Japan OLYMPUS Corporation), ALCYON300 automatic biochemistry analyzer (America Abbott), 756M C UV-visible spectrophotometer (Shanghai Precision Instrument Factory) and GL-20G high-speed refrigerated centrifuge (Shanghai Anting).

Animal grouping

Four days after the rats were fed and adapted; they were selected by 20 min/day in three days. The disqualified that could not get adapted to swimming training were removed. The left were classed into six groups by random number table. The control group (C group) had 12 rats; moderate training group (M group) 12 rats; overtraining group (OM group) 24 rats; epimedium + overtraining group (EOM group) 16 rats; OPC + overtraining group (OPCOM group) 16 rats; epimedium + OPC + overtraining group (EOPCOM group) 16 rats. They were trained to swim for 56 days. In the process, they were fed by gavage on a daily basis, Group EOM with boiled epimedium by the doze of 6.01 g/kg, 5 mL/kg in volume; OPCOM group with OPC solution by the doze of 50 mg/kg, 5 mL/kg in volume; EPCOM group with boiled epimedium by the doze of 6.01 g/kg, 5 mL/kg in volume and OPC solution by the doze of 50 mg/kg, 5 mL/kg in volume, and the other groups with normal saline solution of the same amount.

Training and testing program

C group was raised regularly without interference or training. M group was subjected to swimming of moderate intensity. Swimming was conducted six days every week and once a day. C Group swam 20 minutes the first time and the time was gradually increased later. When the first week ended, the swimming time was increased to 60 minutes; by the end of the second week, the swimming time was lifted to 90 minutes; by the end of the third week, 120 minutes were reached. For the left five weeks, this amount of training was maintained. The other groups followed the same program for the first three weeks, but from the fourth week on, they were given training of high intensity. The rats swan with load until they got exhausted. They went under for ten seconds, which was defined as exhaustion. The load was 0.5% of their weight from week 1 to week 3, 1% for the fourth week, and 2% for the fifth week. The training was done once every day for six weeks. From the seventh week, the training was done in the morning and in the afternoon. In week 7

Table 1. Tubular damage score in various groups of rats (n = 10, $\bar{x} \pm s$)

Groups	Tubular damage score (scores/HP)
C group	2.44 ± 1.37
M group	3.05 ± 1.45[4)]
OM group	12.45 ± 1.79[2)]
EOM group	8.36 ± 1.36[2,3)]
OPCOM group	8.22 ± 1.15[2,3)]
EOPCOM group	7.57 ± 1.69[2,3)]

Note: In comparison with C group, [1)]represent $P < 0.05$, [2)] represent $P < 0.01$; compared with OM group, [3)]and [4)]represent $P < 0.05$ and $P < 0.01$, respectively.

Table 2. Blood urea nitrogen (BUN) and serum creatinine (SCr) levels in plasma (n = 10, $\bar{x} \pm s$)

Groups	BUN (mmol.L^{-1})	SCr (μmol.L^{-1})
C group	8.34 ± 1.11	31.32 ± 10.34
M group	9.42 ± 1.09[1,4,5)]	47.14 ± 10.12[1,4,5)]
OM group	14.19 ± 1.16[2)]	78.64 ± 10.28[2)]
EOM group	10.08 ± 1.11[2,3)]	61.14 ± 10.32[2,3,5)]
OPCOM group	10.65 ± 1.19[2,3)]	62.15 ± 10.46[2,3)]
EOPCOM group	9.47 ± 1.03[1,4,5)]	51.87 ± 10.94[1,4,5)]

Note: In comparison with C group, [1)]represent $P < 0.05$, [2)]represent $P < 0.01$;compared with OM group, [3)]and [4)] represent $P < 0.05$ and $P < 0.01$; comparison with OPCOM group, [5)]and [6)]represent $P < 0.05$ and $P < 0.01$, respectively.

and 8, the training was done in the morning, in the afternoon and in the evening respectively all with the load 5% of their weight. By the end of week 8, C group and M group grow normally without accidental deaths. The other groups suffered from a higher mortality because of the load to their tails and exhaustion. The number of the remaining rats for OM group, EOM, OPCOM and EOPCOM were 14, 12, 12 and 15 respectively. 10 were taken out of each group randomly for the experiment and the others were discarded.

Index determination

The rats of each group were anesthetized with ether 24 hours after their final swimming training. Take the blood from their carotid artery and add anticoagulant sodium citrate solution. Put the kidneys in water at 37°C for 30 minute and centrifuge at 4°C 3000 r/min for 10 minutes so as to separate and prepare serum which was placed in the refrigerator at -20°C. Remove the two kidneys instantly, from which the fascia was scaled, and then place and wash them in the prepared cool saline water. Observe the size, color, and texture. Separate the left kidney, take 0.5 g pole kidney tissue, and grind it with 1.5 mL saline water of below 1°C at 12 000 r/min into 10% kidney tissue homogenates. Part of it was stabilized with 4% glutaraldehyde to be ultrastructurally analyzed. Serum creatinine (Cr) was determined by Jaffe picric acid method; BUN was determined by di-acetyl oxime method; MDA was measured by colorimetry; SOD was measured by Xanthine oxidase. All the kit came from Nanjing Jiancheng Bioengineering Institute, and the kit number was 20130305. It was used strictly according to the manual instructions.

Statistical treatment

Apply SPSS 12.0 software to statistical treatment. The measurement data was expressed in ($\bar{x} \pm s$); the comparison among groups adopted analysis of variance; $\alpha = 0.05$ and 0.01 was the criteria, and $P < 0.05$ indicates that the discrepancy has statistical significance and $P < 0.01$ indicates that the discrepancy has remarkable significance.

Results

Exercise, epimedium and oligomeric proanthocyanidins' effect on the rats' renal pathological changes

After Scr and BUN were measured, the renal tissues of each group were made into paraffin section, stained with HE and observed through optical microscopes. Renal pathological changes were measured against Pallers standard [8]. Five visual fields were chosen at random under 400 times high-power optical microscopes and 10 tubules were chosen from each field for evaluation. (1) Tubules dilated distinctly, and the cells were flat, which was 1 point. (2) Brush-shaped injury was 1 point, and dislocation was 2 points. (3) Membrane bullae were 1 point, and cytoplasmic vacuole was 1 point. (4) Interstitial edema was 1 point. (5) The detached dead cells in the cavity of tubules did not become casts or fragments, and that was 1 point; if they did, that was 2 points. The evaluation of tubules was measured double-blind by two technicians and the average was adopted. C and M group demonstrated normal renal tissues without congestion, degeneration or edema. There was no casts in tubules. Congestion,

Table 3. Comparison of rat kidney homogenate SOD activity and MDA content in each group (n = 10, $\bar{x} \pm s$)

Groups	SOD (U.mgprotmol^{-1})	MDA (U.mgprotmol^{-1})
C group	104.54 ± 2.01	10.01 ± 1.59
M group	94.03 ± 1.95[1,4,5)]	15.84 ± 1.72[1,4,5)]
OM group	60.92 ± 1.24[2)]	35.78 ± 1.68[2)]
EOM group	83.78 ± 1.13[2,3)]	19.98 ± 1.55[2,3)]
OPCOM group	87.24 ± 1.19[2,3)]	18.74 ± 1.64[2,3)]
EOPCOM group	93.42 ± 1.23[1,4,5)]	16.19 ± 1.65[1,4,5)]

Note: In comparison with C group, [1)]represent $P < 0.05$, [2)]represent $P < 0.01$; compared with OM group, [3)]and [4)]represent $P < 0.05$ and $P < 0.01$; comparison with OPCOM group, [5)] and [6)]represent $P < 0.05$ and $P < 0.01$, respectively.

tubular epithelial cell edema, vacuolar degeneration and cavity expansion could be found in OM group, and a small amount of exfoliated villi, epithelial cells and casts stayed in the cavity. The other groups suffered less severe histopathological changes in comparison with OM group. Though mild tubular epithelial cell edema, vacuolar degeneration, and cavity expansion could be seen, there were no protein casts or cellular casts. Tubules of each group were graded by Paller ratings. There was no significant deviation between C and M group ($P > 0.05$), but C and M group were clearly lower than the other groups ($P < 0.01$); EOM, OPCOM, EOPCOM group were significantly lower than OM group ($P < 0.05$), and glomeruli in these groups demonstrated no significant pathological changes (**Table 1**).

Effect of exercises, epimedium and oligomeric proanthocyanidins on the rats' serum BUN and SCr

Concluded from **Table 2**, in terms of SUN and creatinie levels, M group ($P < 0.05$), OM group ($P < 0.01$), EOM group ($P < 0.01$), OPCOM group ($P < 0.01$), EOPCOM group ($P < 0.05$) were higher than C group; M group ($P < 0.01$), EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), EOPCOM group ($P < 0.01$) were lower than OM group; EOPCOM group ($P < 0.05$) was lower than OPCOM group, EOM group and OPCOM group showed no significant deviation ($P < 0.05$).

Effect of exercises, epimedium and oligomeric proanthocyanidins on SOD activity and MDA content in the rats' kidney tissue homogenates

Seen from Table 3, in terms of SOD activity, M group ($P < 0.05$), OM group ($P < 0.01$), EOM group ($P < 0.01$), OPCOM group ($P < 0.01$), and EOPCOM group ($P < 0.05$) were lower than C group; M group ($P < 0.01$), EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), and EOPCOM group ($P < 0.01$) were higher than OM group; EOPCOM group ($P < 0.05$) was higher than OPCOM group; EOM group and OPCOM group showed no significant deviation ($P < 0.05$). In terms of MDA content, M group ($P < 0.05$), OM group ($P < 0.01$), EOM group ($P < 0.01$), OPCOM group ($P < 0.01$), and EOPCOM group ($P < 0.05$) were higher than C Group; M group ($P < 0.05$), EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), EOPCOM group ($P < 0.01$) were lower than OM group; EOPCOM group ($P < 0.05$) was lower than OPCOM group; EOM group and OPCOM group demonstrated no significant deviation ($P > 0.05$).

Discussion

The kidney is an organ of high perfusion and very sensitive to reperfusion. When ischemia lasts for a certain time and reperfusion occurs, sometimes it does not lead to structural and functional recovery of tissues. Instead it aggravates functional and structural damage to the kidney [9, 10]. This is the so called renal ischemic-reperfusion injury. When the organism is stale, renal blood flow can stay stable through self-regulation. During intense sports, renal blood flow changes under the influence of nerves and body fluids. Because of the redistribution of blood flow, muscular blood flow increases, while renal blood flow decreases sharply and this decrease grows more conspicuous as the duration and intensity of exercises progress. Renal ischemic reperfusion (I/R) injury is a complicated pathological and physiological process and its mechanism is yet to discern. But oxygen free radicals play an important role in the generation, development of I/R process [9, 11]. In normal circumstances, the body produces a small amount of radicals. However, due to the radical-inactivating enzyme system, radicals are quickly removed and no damage is done. In sports-induced renal ischemic-reperfusion injury, reperfusion injury is more remarkable. During the renal ischemic period, a lot of ATP is consumed, and AMP, adenosine, inosine, and hypoxanthine increase. Under normal physiological conditions, hypoxanthine is transformed into xanthine and eventually into uric acid by xanthine dehydrogenase and NAD+,

and no oxygen free radicals are produced. But when oxygen is lacking, hypoxanthine is transformed into xanthine oxidase rather than be oxidized into xanthine. Consequently, lots of hypoxanthines accumulate. When reperfusion brings much oxygen, many active oxygen radicals are generated. They are also produced in other ways. For example, lots of polymorphonuclear leukocytes and catecholamine in kidney tissues produce radicals in auto-oxidation when reperfusion happens; mitochondria cannot supply adequate electrons to restore oxygen free radicals to H_2O_2; a shortage of SOD brings about accumulation of radicals. Radicals-induced tissue injury is primarily achieved through lipid peroxidation. In renal cell unit membrane (cells, mitochondria, lysosomal, endoplasmic reticulum), a lot of unsaturated fatty acid can react with oxygen radicals and produce lipid peroxide radicals, which further trigger peroxidation of unsaturated fatty acid and lead to an endless vicious circle. As a result, membrane function is changed, and cells and organelles damaged [12, 13]. In addition, MDA, a decomposition product of oxygen free radicals and lipid peroxides, interferes with metabolism of protein, sugar and nucleic acid, retards the activity of enzyme, and wrecks the functions of nucleic acid template and tissue structure. In exercise, the generation of renal radicals can cause peroxidation of membrane lipids, which consequently leads to alteration of membrane liquidation, fluidity and transparency and membrane dysfunction. Severe damage to membrane structure, mitochondria membrane in particular, affects cellular metabolism and function and physiological function of the whole organ [14, 15]. In a state of ischemia, renal tubular endothelial cells are the primary source of OFR, and among endothelial cells, mitochondria are the major part to produce OFR. Body produced SOD, CAT and the like can remove ORF quickly [16, 17]. OFR is very powerful in oxidation activity. It can react with various cellular components and lead to lipid peroxidation of polyunsaturated fatty acids in biomembranic phospholipids. For one thing, membrane enzyme is damaged or activated; for another, membrane fluidity declines and transparency rises, and meanwhile, many toxic decomposition products are generated to undermine proteins, nucleic acids and chromosomals, and intercellular contents and eventually damage cells [18, 19]. Peroxide lipids are ultimately decomposed into MDA. The extent of peroxidation indirectly reflects the injury extent of cells caused by oxygen free radicals [20]. Therefore, determining MDA content can assess the level of OFR and the intensity of lipid peroxidation in the kidney. SOD, a metalloproteinases, negatively charged, is one of the most important antioxidases in the body. Its major active substrate is superoxide anion radical (0-2) in OFR which can interrupt the chain reaction of lipid peroxidation and thus protect cells from damage [21]. It plays an important role in balancing bodily oxidation and antioxidation. When renal blood is lacking, lipid oxidation becomes active, and more MDA is produced, when SOD decreases because of consumption. So SOD activity reflects indirectly organic capability of removing radicals. Creatinine and urea nitrogen are the catabolites of muscles and proteins. They are discharged from the kidney through blood circulation. Their concentration is decided by glomerular filtration capacity. When renal parenchyma is damaged, glomerular filtration rate fall below critical point, concentration of the two rises considerably [22].

The results of the experiment reveal that 8 weeks of overtraining resulted in evident histopathological change in renal tissues of rats; BUN and serum creatinine mount (OM group ($P < 0.01$), EOM group ($P < 0.01$), OPCOM group ($P < 0.01$), EOPCOM group ($P < 0.05$) higher than C group). SOD activity declines (OM group ($P < 0.01$), EOM group ($P < 0.01$), OPCOM group ($P < 0.01$), EOPCOM group ($P < 0.05$) lower than C group). MDA content rises, (OM group ($P < 0.01$), EOM group ($P < 0.01$), OPCOM group ($P < 0.01$), EOPCOM group ($P < 0.05$) higher than C group). The results show that overtraining led renal ischemic reperfusion injury to the rats and renal function is greatly damaged. But in terms of histopathological change of renal tissues, EOM group, OPCOM group, and EOPCOM group ($P < 0.05$) remarkably lessened in comparison with OM group. In terms of BUN and serum creatinine content, EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), EOPCOM group ($P < 0.01$) are lower than OM group; in terms of SOD activity, EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), EOPCOM group ($P < 0.01$) are higher than OM group; in terms of MDA content, EOM group ($P < 0.05$), OPCOM group ($P < 0.05$), EOPCOM group ($P < 0.01$) are lower than OM group. The results also prove that epimedium

and OPC can effectively remove radicals, alleviate renal lack of blood and oxygen, inhibit lipid peroxidation, enhance SOD activity, effectively reduce renal ischemic reperfusion injury and protect the kidney. The possible mechanism may be the following. (1) Epimedium TFE can erase over-produced radicals so as to prevent their damage to organic cells, activate SOD activity, reduce LPO content, inhibit accumulation of lipofuscin in tissue cells. (2) Epimedium contains Vc, polyphenols and other compounds which have multiple alkyls that can restore superoxide anion radicals by self-oxidation, enhance antioxidase activity, and help get rid of radicals during the rats' exercise [23]. (3) EPS can generate fresh organic radicals through self-oxidation so as to strike a balance between radicals polysaccharide produces and those polysaccharide erases or even surpass this balance, and help remove radicals when the rats are trained [24]. (4) Magnoflorine, the major element of epimedium, carries N+ that attracts hydroxyl radicals and reduces their reactivity [25]. (5) OPC is a crucial antioxidant vitamin which can upgrade SOD activity, erase oxygen free radicals, inhibit lipid peroxidation, and boost organic antioxidation capacity to lighten ischemic reperfusion injury of cells and organs. In addition, when the three treatment groups are compared, in terms of BUN and serum creatinine, EOPCOM group ($P < 0.05$) is lower than OPCOM group, and there is no significant deviation between EOM group and OPCOM group ($P > 0.05$); in terms of SOD activity, EOPCOM group ($P < 0.05$) is higher than OPCOM group, and EOM group and OPCOM group demonstrate no significant difference ($P > 0.05$); in terms of MDA content, EOPCOM group ($P < 0.05$) is lower than OPCOM group, and EOM group and OPCOM group show no significant difference ($P < 0.05$). The results indicate that epimedium and OPC exert similar effect on renal ischemic reperfusion injury. The joint application of the two reduces BUN and serum creatinine more effectively than only one item is used. SOD activity is boosted, which suggests that the combination of the two may be more favorable to protecting the kidney injured by ischemic reperfusion.

Conclusion

Epimedium and OPC are both helpful in protecting the kidney injured by ischemic reperfusion. The protection may come from intensifying SOD activity, erasing radicals, lighten lipid peroxidation. In terms of therapeutic effect, joint effect of the two is much better than the single use of them. Epimedium exerts similar effect to OPC, but the dose of the two may be at work as well. As for the relation between the dose and effect, further study is needed.

Disclosure of conflict of interest

None.

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