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Protective effects of *Ginkgo biloba* extract on cultured rat cardiomyocytes damaged by H_2O_2

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KEY WORDS *Ginkgo biloba*; oxygen; free radicals; myocardium; cultured cells; hydrogen peroxide

ABSTRACT

AIM: To investigate the influence of *Ginkgo biloba* extract (*Gb*E) on cardiomyocytes damaged by H_2O_2. **METHODS**: Cultured rat cardiomyocytes were divided into 3 groups randomly: control group; H_2O_2 (2.5 mmol·L^{-1}) group; H_2O_2 2.5 mmol·L^{-1} + *Gb*E 150 mg·L^{-1} group. The cardiomyocytes were cultured in MEM (Eagle's) at 37 ℃ in the presence of 5 % CO_2 for 4 h. Lactate dehydrogenase (LDH) was assayed by colorimetric method. Lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances. Ultrastructure was viewed under transmission electron microscope. **RESULTS**: Compared with the control group, LDH leakage and malondialdehyde (MDA) content increased in H_2O_2 group. LDH increased from (2166 ± 247) U·L^{-1} to (5180 ± 648) U·L^{-1}. MDA increased from (3.5 ± 0.2) nmol/10^6 cells to (7.2 ± 0.4) nmol/10^6 cells ($P < 0.01$). The ultrastructure was damaged seriously. *Gb*E inhibited the increase of LDH leakage and MDA content induced by H_2O_2. In this group, LDH decreased from (5180 ± 648) U·L^{-1} to (3496 ± 386) U·L^{-1}, MDA decreased from (7.2 ± 0.4) nmol/10^6 cells to (4.8 ± 0.9) nmol/10^6 cells ($P < 0.01$). Ultrastructure of cells was also protected by *Gb*E. **CONCLUSION**: *Gb*E protected the cardiomyocyte against H_2O_2 injury, the protective action was attributed to its antiperoxidative effect.

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INTRODUCTION

Ginkgo biloba has been a staple of Chinese medicine for thousands of years, being recommended for coughs, asthma, and acute allergic inflammation. *Ginkgo biloba* extract (*Gb*E) can react with free oxygen radicals such as $O_2^{\cdot}$, ·OH, 2, 2-DPPH *in vitro*[1]. *Gb*E had a superoxide dismutase-like activity[2]. However, the report of the effect of *Gb*E on cultured cells damaged by free oxygen radicals has not been found. In the present study, the influence of *Gb*E on cultured cells damaged by H_2O_2 was studied.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (Certificate № 2-22-11) aging 2 – 3 d (n = 60) were obtained from Experimental Animal Center of Shanghai Medical University.

Drug *Gb*E was the product of Willmar Schwabe Pharmaceutical Factory, Germany. (lot X940261, 3.5 g·L^{-1}). Per ampoule was standardized to contain *Ginkgo* flavoneglycosides 4.2 mg. Injection solution 1 mL contained sorbitol 40 mg and 3.5 % ethyl alcohol by volume. *Gb*E contained 24 % of flavonoid glycosides[3], the aglycone of which was a flavonol (including quercetin, kaempferol, and isorhamnetine), 6 % of terpene lactones (including ginkgolides A, B, C, J, and bilobalide), and 70 % of other substances (proanthocyanidins, organic acids, sugars, *etc*).

Preparation of cardiomyocytes Isolation of cardiomyocyte from rat heart was performed[4], the cardiomyocytes were grown in MEM (Eagle's) culture medium containing 20 % fetal bovine serum. The cell density was adjusted to 5 × 10^6 cells·L^{-1}, and 1 mL of the suspension was pipeted into each well of 24-well culture plates. Cells were incubated at 37 ℃ in 5 % CO_2 atmosphere for 4 d.

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Experimental design Cardiomyocytes were used to determine the effect of *Gb*E on oxidation induced by H_2O_2. Solution of H_2O_2 was prepared in PBS buffer. Cardiomyocytes were incubated in medium containing H_2O_2 2.5 mmol · L^{-1} with or without *Gb*E 150 mg · L^{-1} at 37 ℃ in 5 % CO_2 for 4 h. The cells were viewed under an inverted phase contrast microscope and photographed using a camera attachment.

LDH assay The supernatant was collected from every well for LDH assay. LDH was measured by colorimetric method[5].

Lipid peroxidation Malondialdehyde (MDA) in membrane was measured. After supernatant was collected, the adherent cells were then dispersed with trypsin/edetic acid (0.1 %/0.04 %) in D-Hanks' solution for 2 min and were ultrasonicated. The cell fragment suspension was collected and the extent of lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances[6].

Ultrastructure Cardiomyocytes were cultured in culture flask under the same circumstance, after treatment with H_2O_2 + *Gb*E, the cells were fixed with 2.5 % glutaraldehyde, then 70-nm ultra-thin sections of cells were made and viewed under transmission electron microscope (TEM).

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed by t-test.

RESULTS

Effects of *Gb*E on beat and morphologic change of cardiomyocytes injuried by H_2O_2 In control culture wells, cardiomyocytes beated synchronously and their appearances were normal. Cells incubated with H_2O_2 2.5 mmol · L^{-1} stopped beating, and had a morphologic change: cell pseudopod decreased or contracted, cells tended to be round and granule in cytoplasm increased. After incubation with H_2O_2 2.5 mmol · L^{-1} + *Gb*E 150 mg · L^{-1}, cells stopped beating too, but had no morphologic change (Fig 1).

Effect of H_2O_2 and *Gb*E on lactate dehydrogenase (LDH) leakage and lipid peroxidation When cardiomyocytes were incubated without H_2O_2, LDH leakage maintained at a low level, (2166 ± 247) U · L^{-1}. H_2O_2 increased LDH by 239 % *vs* control

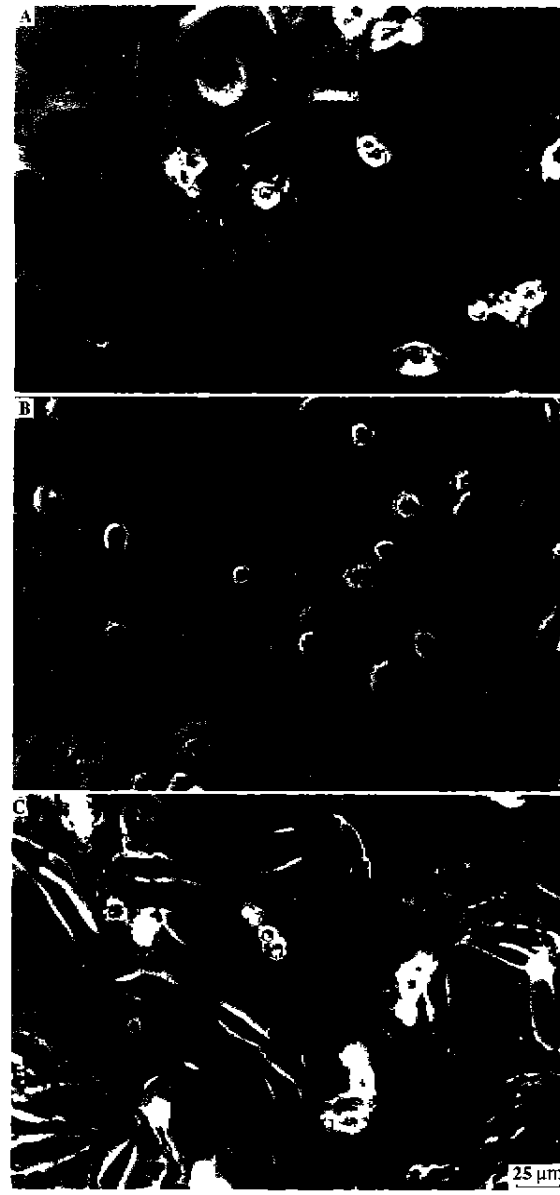

Fig 1. Micrographs of cultured cardiomyocytes. × 400. A: Control group. B: H_2O_2 group. C: *Gb*E group.

($P < 0.01$). Incubation with *Gb*E 150 mg · L^{-1} resulted in a decline of LDH release in H_2O_2 treated cells (Tab 1).

Effects of *Gb*E on lipid peroxidation of cardiomyocytes were measured by the content of MDA. H_2O_2 increased MDA *vs* control group ($P < 0.01$). When cells were pretreated with *Gb*E 150 mg · L^{-1}, MDA was reduced markedly (Tab 1).

Effect of *Gb*E on ultrastructure of cardiomyocytes injuried by H_2O_2 Cell ultrastructure in

Tab 1. Effect of *Gb*E on cardiomyocyte injuried by H_2O_2. $n = 15$ experiments, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control group, $^fP < 0.01$ *vs* H_2O_2 group.

	LDH (U·L^{-1})	MDA (nmol/10^6 cells)
Control	2 166 ± 247	3.48 ± 0.24
H_2O_2	5 180 ± 648^c	7.2 ± 0.4^c
H_2O_2 + *Gb*E	3 496 ± 386^f	4.8 ± 0.9^f

control group was normal. The ultrastructure of cardiomyocyte demonstrated regular arrangement of myofibril and clear mitochondria structure. The cell membrane was integrated. The nucleus was ellipse and chromatin distributed homogeneously in the nucleus. After exposure to H_2O_2, cell membrane was unintegrated, volume of cell organ decreased. Mitochondrial structure was not clear, the density of its crista was diminished. Myofibrils were broken down. *Gb*E diminished damage at mitochondrial crista and matrix induced by H_2O_2, and distended the sarcoplasmic reticulum. The membrane was integrated, almost normal (Fig 2).

DISCUSSION

H_2O_2 *in vitro* decreased ATP concentration and mediate cell injury[7,8]. In our present study, cultured cardiomyocytes were damaged seriously by H_2O_2, too.

In our experiment, *Gb*E decreased LDH leakage from cardiomyocyte and MDA content caused by H_2O_2, and prevented the injury of cell structure. But *Gb*E 150 mg·L^{-1} could not keep the cells injuried by H_2O_2 2.5 mmol·L^{-1} beating. It indicated that not all of the funtion injury caused by H_2O_2 2.5 mmol·L^{-1} could be liminated by *Gb*E 150 mg·L^{-1}. However, *Gb*E 150 mg·L^{-1} was able to prevent the further injury to the cell structure. The potence contributed to *Ginkgo* flavonoids in *Gb*E. In order to find the best protective result, more dosage of *Gb*E should be used in our present experiment.

In conclusion, *Gb*E protected cardiomyocyte against H_2O_2 injury. The protective action may be attributed to its antiperoxidative effect, suggesting that *Gb*E should prevent and treat free radical-induced disorders.

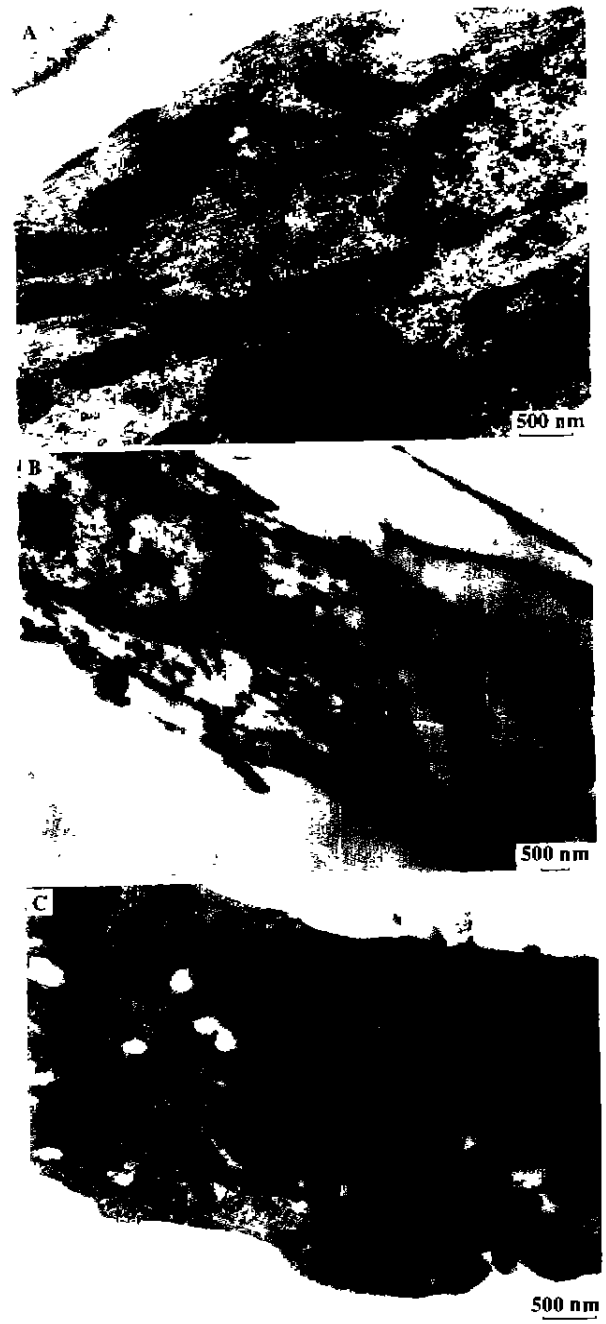

Fig 2. Electron micrographs of cultured cardiomyocytes. Lead citrate, uranyl acetate double stain. A (×20 000): Control group. B (×12 000): H_2O_2 group. C (×20 000): *Gb*E group.

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REFERENCES

1 Pincemail J, Dupuis M, Nasr C, Hans P, Haag-Berrurier M, Anton R, *et al*. Superoxide anion scavenging effect and superoxide dismutase activity of *Ginkgo biloba* extract. Experientia 1989; 45: 708 - 12.
2 Duke MV, Salin ML. Purification and characterization of iron-containing superoxide dismutase from a eucaryote. *Ginkgo biloba*. Arch Biochem Biophys 1985; 243: 305 - 14.
3 Defeudis FV. *Ginkgo biloba* extract (EGb761): pharmacological activities and clinical applications. Paris: Elsevier; 1991. p 9 - 24; p 61 - 94.
4 Yang YZ, Dyke JW. Coxsackie B-Z virus infection in rat beating heart cell culture. J Virol Methods 1985; 12: 217 - 9.
5 Li JZ. Lactate dehydrogenase assay. In: Ye YW, editor. Clinical laboratory diagnostics. 1st ed. Beijing: The Peaple's Medical Publishing House; 1989. p 598 - 603.
6 Wong YC, Wang XP, Lu YC, Guo ZZ, Shi F. Microdetermination of lipid peroxidation in cells and cell membrane. J Cytobiol 1985; 7: 142 - 3.
7 Zweier JL, Kuppusamy P, Williams R, Rayburn BK, Smith D, Weisfeldt ML, *et al*. Measurement and characterization of postischemic free radical generation in the isolated perfused heart. J Biol Chem 1989; 264: 18890 - 5.
8 Wilson J, Winter M, Shasby DM. Oxidants, ATP depletion and endothelial permeability to macromolecules. Blood 1990; 76: 2578 - 82.

银杏提取物对过氧化氢损伤的培养大鼠心肌细胞的保护作用

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关键词 银杏；氧；自由基；心肌；培养的细胞；过氧化氢

目的：探讨银杏叶提取物（*Gb*E）对过氧化氢损伤的培养心肌细胞的保护作用及其机制. **方法**：比色法测定乳酸脱氢酶活性；戊巴比妥酸法测定细胞内脂质过氧化物含量；透射电镜下观察细胞超微结构. **结果**：过氧化氢导致心肌细胞 LDH 释放从（2166 ± 247）$U \cdot L^{-1}$增至（5180 ± 648）$U \cdot L^{-1}$，MDA 含量从每 10^6 细胞（3.5 ± 0.2）nmol 增至（7.2 ± 0.4）nmol；心肌细胞超微结构受到严重损伤. 加 *Gb*E 使 LDH 释放从（5180 ± 648）$U \cdot L^{-1}$降至（3496 ± 386）$U \cdot L^{-1}$；MDA 生成由每 10^6 细胞（7.2 ± 0.4）nmol 降至（4.8 ± 0.9）nmol 并减轻心肌超微结构的损伤. **结论**：*Gb*E 通过清除氧自由基保护过氧化氢损伤的心肌细胞.

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读者注意

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