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Protective effects of *Ganoderma lucidum* polysaccharides peptide on injury of macrophages induced by reactive oxygen species[1]

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KEY WORDS *Ganoderma lucidum* polysaccharides peptide; reactive oxygen species; macrophages; antioxidants; mitochondria; tert-butylhydroperoxide

ABSTRACT

AIM: To study the protective effects of *Ganoderma lucidum* polysaccharides peptide (GLPP) on the mice peritoneal macrophages injured by reactive oxygen species (ROS), derived from tert-butylhydroperoxide (tBOOH) *in vitro* and *in vivo*. **METHODS:** Mice peritoneal macrophages were injured by ROS, derived from tBOOH. The survival rate of macrophages was measured by MTT assay, and the morphological changes of macrophages were observed under light and electron microscopes. **RESULTS:** GLPP (50, 100, 200 mg/kg, ip for 5 d) could inhibit the foam cell formation and necrosis of macrophages. The survival rate of macrophages was increased. GLPP (3.125, 12.5, 50, 200 mg/L) given to the cultured macrophages brought the same protective effects. Under the electron microscope it was found that GLPP (100 mg/kg, ip, for 5 d) could protect the organelle such as mitochondria against injury by tBOOH. **CONCLUSION:** GLPP had significant scavenging ROS and antioxidant effects.

INTRODUCTION

Ganoderma polysaccharides and *Ganoderma lucidum* polysaccharides peptide (GPP) were extracted from *Ganoderma lucidum* (Leyss ex Fr) Karst. A great deal of experimental evidence has accumulated in the past several decades, suggesting that *Ganoderma lucidum* polysaccharides had wide bioactivities, such as immune modulation and antitumor[1,2], anti-atherosclerosis, anti-diabetes, and anti-aging activities, *etc*[3]. Recently, it was reported that *Ganoderma* polysaccharides had scavenging reactive oxygen species (ROS) effects[4]. The pathophysiological mechanisms of tumor, atherosclerosis, diabetes, and aging were associated with the ROS[5-8]. Mitochondrion was the major site of ROS production and was the major place to be injured by ROS[9]. The function of mitochondrion in macrophage was active. In this paper, we examined whether GLPP could protect the mice

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peritoneal macrophages against the injury by ROS.

MATERIALS AND METHODS

Drug *Ganoderma lucidum* polysaccharides peptide (GLPP) isolated from *Ganoderma lucidum* (Leyss ex Fr) Karst by boiling water was provided by Fuzhou Institute of Green Valley Bio-Pharm Technology. GLPP was hazel colored powder with an average molecular weight of 5.13×10^5 and contained 16 kind of amino acids as follow:

Asp 8.49, Thr 3.58, Ser 3.93, Glu 5.81, Gly 3.50, Ala 3.84, Cys1.06, Val 2.68, Met 5.33, Iso-Leu 0.25, Leu 1.5, Phe 1.99, Lys 3.30, His 1.21, Arg 3.94, Pro1.22 (mg/g). The polysaccharides peptide consisted of rhamnose, xylose, fructose, galactose, glucose with molar ratio of 0.549:3.614:3.167:0.556:6.89 and linked together by β-glycosidic linkages.

MTT, RPMI-1640, and tert-butylhydroperoxide (tBOOH) were purchased from Sigma.

Animals BALB/c male mice (weighing 20 g±2 g) were purchased from the Animal Center of Beijing University Health Science Center (Grade II, Certificate № scxk11-00-0004).

Cell culture BALB/c mice were randomly divided into 4 groups. The mice in the treated group were received GLPP (50, 100, 200 mg/kg, ip). The mice in the control group received 0.9 % normal saline ip for 5 d. On the 7th day, the mice peritoneal macrophages were prepared as described previously[10]. The cells were seeded on 96-well plate at a cell density of 2×10^8/L in RPMI-1640 containing 15 % calf serum. After incubated in 5 % CO_2 atmosphere at 37 ℃ for 4 h, cells were washed with PBS three times. Then tBOOH (0, 0.77, 0.1, 0.22 mmol/L) contained in culture medium was added and incubated for another 24 h.

Macrophages of untreated mice were prepared as above and seeded on 96-well plate. The cells were divided into 5 groups and the macrophages of each group were added with GLPP 0, 3.125, 12.5, 50, 200 mg/L, respectively. After incubated for 24 h, tBOOH 0.1 mmol/L was added and incubated for another 24 h.

MTT assay To determine survival rate of macrophages, MTT assay was performed as previously described[11]. The absorbance was measured in the ELISA plate reader at 540 nm.

Statistics Data were expressed as mean±SD and analyzed by one way ANOVA.

Morphology Male mice were divided into 3 groups. Mice in the treated group were received GLPP (100 mg/kg, ip). Mice in tBOOH injury group and control group received 0.9 % saline for 5 d. On the 7th day, the mice were sacrificed and macrophages were prepared as above then the macrophages were seeded on 6-well plate. The macrophages of the treated group and tBOOH group were injured with tBOOH (0.1 mmol/L for scan electron microscope or 0.01 mmol/L for transmission electron microscope) for 24 h. The macrophages of control group were treated with equal volume of RPMI-1640 medium. Then the macrophages were harvested, fixed, and observed.

RESULTS

Survival rate of macrophages The survival rate of macrophages exposed to tBOOH (0, 0.1, 0.22 mmol/L) for 24 h was increased respectively by GLPP (100, 200 mg/kg) *in vivo* (Tab 1). GLPP (3.125, 12.5, 50, 200 mg/L) also increased the survival rate of macrophages *in vitro* (Tab 2).

Tab 1. Protective effects of *Ganoderma* polysaccharides peptide ip on the survival rate of macrophages incubated with tBOOH for 24 h. *n*=6. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* GLPP 0 mg/kg.

		Survival rate/%		
tBOOH/mmol·L^{-1}	0	0.77	0.1	0.22
GLPP 0 mg·kg^{-1}	100	75±16	72.2±2.0	35±5
50 mg·kg^{-1}	100	80±3	63±6	58±6
100 mg·kg^{-1}	100	88±7	92±9^c	96±15^c
200 mg·kg^{-1}	100	77±13	78±8	74±17^b

Morphology Under the light microscope, above half of the macrophages appeared asteroid and there was no or little phagocytic granule before treatment with

Tab 2. Protective effects of GLPP on the survival rate of cultured macrophages incubated with tBOOH (0.1 mmol/L) for 24 h. *n*=4. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

Concentration of GLPP/mg·L^{-1}	Survival rate/% Before-treatment	After-treatment
0	100	29.4±1.9
3.125	100	58±15^{b}
12.5	100	52±6^{b}
50	100	61±25^{b}
200	100	90±16^{c}

tBOOH. After treated by tBOOH, macrophages in control group became round and the granule increased. The injury of macrophages increased following the increase of the concentration of tBOOH. Cell appeared foam and lipid droplets were found. Necrosis was found in part of the macrophages. Cell debris could be seen and the number of macrophages decreased. The injury of macrophages treated with GLPP decreased. Most of the macrophages stills appeared asteroid and the granule and the lipid droplet of macrophages were reduced. GLPP showed protective effects on macrophages against injury by tBOOH *in vivo* (Fig 1A, B, C). GLPP also showed the protective effects on macrophages against injury by tBOOH *in vitro*.

Scan electron microscope showed that in tBOOH-injured group, the microvilli of the macrophages were short and scare, with spot like distribution and part of the membrane even became smooth. The microvilli were injured slightly or kept intact in the GLPP-treated group (Fig 2A, B, C). Under the transmission electron microscope, injured mitochondria were obviously observed in tBOOH-treated group. There appeared huge mitochondria and some of the mitochondria become stratified. Rough endoplasmic reticulums were reduced in numbers. Many lipid droplets appeared and a lot of macrophages showed foamy change. In GLPP-treated group, the injury of mitochondria was decreased. The cristae of the mitochondria were slightly disorganized but the structure was still intact. Endoplasmic reticulums were tubiform and vesiculiform. There was little lipid droplet and foamy changes in the structure of microphages cytoplasm (Fig 3A, B, C). GLPP produced protective macrophages against injury by tBOOH.

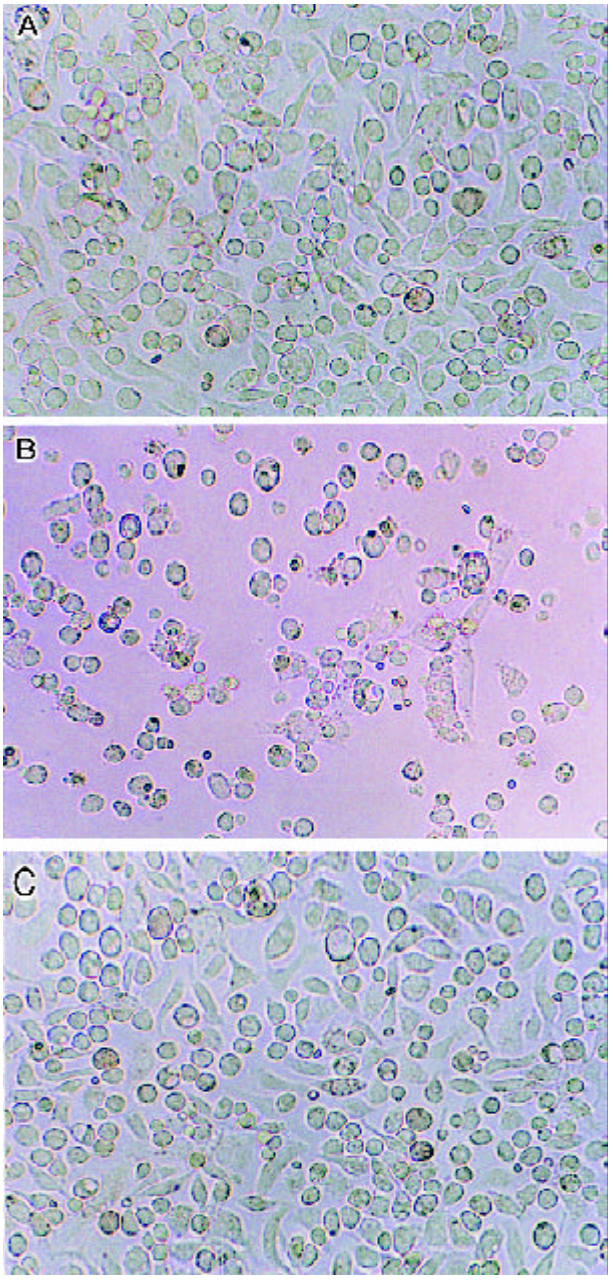

Fig 1. Effects of GLPP on the mice macrophages injured by tBOOH. Light microscope, ×400. A: Control (without treatment with tBOOH); B: treated with tBOOH; C: GLPP-treated group.

DISCUSSION

tBOOH, membrane-permeant oxidant has been extensively used as a model of oxidative in different

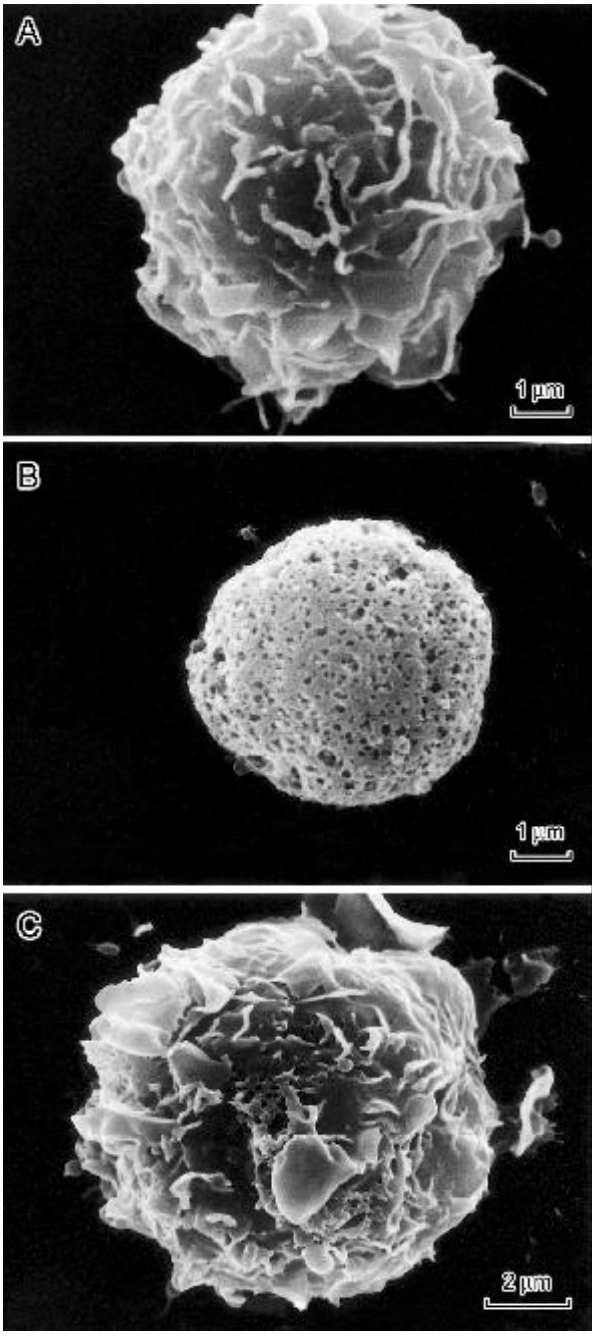

Fig 2. A macrophage incubated with tBOOH (0.1 mmol/L) for 24 h was observed under the scan electron microscope. A: Long microvilli were observed in control group (×11 000); B: Membrane of macrophages became smooth in the tBOOH-treated group (×11 000); C: A few of microvilli of macrophages were slightly short in GLPP-treated group (×8000).

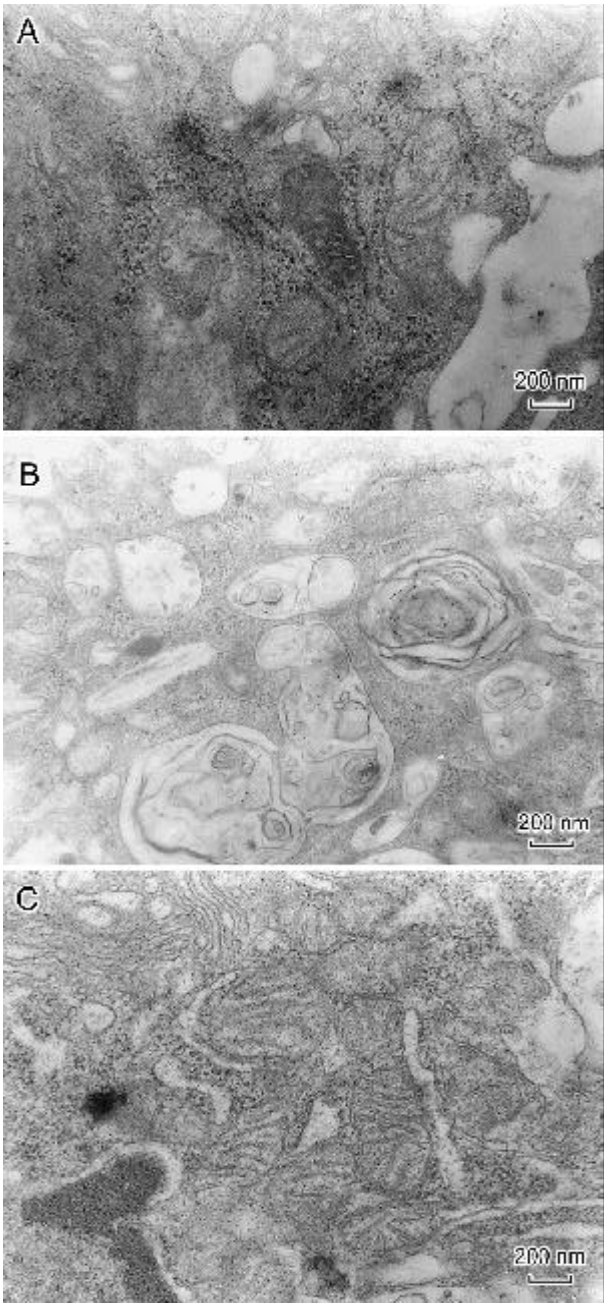

Fig 3. Structure of microphages incubated with tBOOH (0.01 mmol/L) for 24 h was observed under transmission electron microscope. A: The structure of mitochondria was normal in control group (×30 000); B: Structure of mitochondria became stratified in the tBOOH-treated group (×25 000); C: The cristae of mitochondria was slightly disorganized or unchanged in GLPP-treated group (×30 000).

system[12]. In this study, we used it as an oxidant to produce oxidative damage stress on macrophages. The result showed that GLPP could prevent tBOOH-induced cell oxidative injury *in vivo* and in *vitro*. GLPP increased the survival rate of macrophages injured by tBOOH. The morphology change under the light microscope and electron microscope showed that GLPP could protect the cell organelles such as mitochondria and endoplas-

mic reticulums against injury. The mitochondria, which were the major site producing ROS and also subjected to great injury by ROS, were significantly protected. It suggested that GLPP had potential scavenging ROS and antioxidant effects.

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灵芝多糖肽对氧自由基损伤巨噬细胞的保护作用[1]

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关键词 灵芝多糖肽；活性氧自由基；巨噬细胞；抗氧化剂；线粒体；叔丁基氢过氧化物

目的：研究灵芝多糖肽(GLPP)在离体和整体水平对氧自由基(ROS)(tBOOH为氧化剂)损伤巨噬细胞的保护作用．方法：以tBOOH为氧化剂损伤小鼠腹腔巨噬细胞，以MTT法分析小鼠巨噬细胞存活率，在光镜和电子显微镜下观察细胞的形态改变．结果：GLPP 50，100，200 mg/kg腹腔注射5天，能抑制巨噬细胞膜样变性和坏死，细胞存活率提高．在培养的巨噬细胞中加入GLPP 3.125，12.5，50，200 mg/L，产生相似的保护作用．电镜观察发现，GLPP(100 mg/kg)腹腔注射5天可保护细胞器如线粒体免受tBOOH的损伤．结论：GLPP有显著的清除氧自由基和抗氧化作用．

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