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Effect of *Ganoderma lucidum* Polysaccharide on Antioxidative Ability of Rats with Myocardial Injury Induced by Doxorubicin

Fan Xu[1], Xiao Li[2], Qingshan Li[1], Xiao Xu[3], Wenxin Li[2], Xiaolei Yu[1*]

[1]Departments of Oncology, Affiliated Hospital of Chengde Medical College, Chengde, China
[2]Departments of Radiology, Affiliated Hospital of Chengde Medical College, Chengde, China
[3]Departments of Pharmacy, Affiliated Hospital of Chengde Medical College, Chengde, China
Email: *28574060@qq.com

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Abstract

Objective: The objective is to investigate the effect of *Ganoderma lucidum* polysaccharide (GLPS) on the oxidative stress induced by doxorubicin (DOX) in cardiomyocytes. **Methods:** SD rats were divided into control group, DOX group, GLPS low dose and high dose + DOX group. SOD and MDA levels in myocardial tissue were detected in each group. The expression of related proteins in each group was detected by Western blot. **Results:** GLPS can increase the SOD level and decrease MDA caused by DOX ($p < 0.05$). GLPS can reduce the increase of p53 content caused by DOX ($p < 0.05$), and up regulate the antioxidant enzyme HO-1 ($p < 0.05$), MDM2 ($p < 0.05$) and Nrf2 ($p < 0.05$). **Conclusion:** Ganoderma polysaccharide can improve the oxidative stress injury of myocardial tissue induced by doxorubicin and play a protective role in myocardial tissue.

Keywords

Doxorubicin, *Ganoderma lucidum* Polysaccharide, Myocardial Tissue, Antioxidant Capacity

1. Introduction

Ganoderma lucidum is a kind of fungus with a history of more than 2000 years. It is known as the "four fairy Grasses" with ginseng, *Polygonum multiflorum* and *Cordyceps sinensis*. Ancient Chinese medical scholars believed that *Ganoderma lucidum* had the function of "strengthening the body and strengthening the foundation". It contains about 400 different active substances. *Ganoderma lucidum* polysaccharide is the main functional component, which has the func-

tions of scavenging oxygen free radicals, anti myositis and protecting the heart [1]. However, there are few reports on reducing myocardial injury caused by drugs.

Doxorubicin is a representative anthracycline drug, which plays an important role in the clinical treatment of tumors, but it has cardiotoxicity. The possible mechanism is that cardiomyocytes are damaged by oxidative stress, resulting in the imbalance between intracellular antioxidant and pro-oxidative [2]. We can reduce cardiotoxicity by enhancing cellular antioxidant capacity. Antioxidant drugs such as coenzyme Q10, vitamin C and E are used to prevent heart injury caused by doxorubicin, but the effect is not good; at present, there is no oral drug recognized to effectively inhibit the cardiotoxicity of anthracyclines. Therefore, it is significant to find a convenient and effective drug to combat its cardiotoxicity. In this study, we used *Ganoderma lucidum* polysaccharide to intervene the myocardial injury induced by doxorubicin in rats in order to explore its possible mechanism.

2. Materials and Methods

2.1. Rats and Materials

40 male SD rats (weighing 220 - 250 g) are purchased from Beijing Kangtai Heyuan biological company (license No. scxk Jing 2012-0001). Horseradish peroxidase labeled Goat anti-rabbit IgG secondary antibody, p53 protein, mouse double micro gene 2 (MDM2), heme oxygenase 1 (HO-1), nuclear factor E-2 related factor 2 (Nrf2), β-actin antibodies are purchased from Proteintech (Wuhan, China). BCA protein concentration determination kit, serum malondialdehyde (MDA) kit, oxide dismutase (SOD) kit, doxorubicin and *Ganoderma lucidum* polysaccharide (purity 99%) are purchased from Kangtai Heyuan biological company (Beijing, China), EuroblotmasterII and EUROLineCamera (Beijing, China).

2.2. Rat Grouping and Model Preparation

Forty male SD rats were randomly divided into four groups with 10 rats in each group: control group, DOX group, GLPS low dose + DOX group and GLPS high dose + DOX group. Model preparation method [3]: dissolve DOX in 5 mL water for injection, add 0.9% normal saline, configure 1 ml normal saline to contain 0.8 mg DOX, and store it in dark and low temperature for standby. Dox group, GLPS low dose + DOX group, GLPS high dose + DOX group were injected with DOX at 2.5 mg/kg intraperitoneally from the second day of the experiment, once every other day, a total of 6 times. The control group was intraperitoneally injected with the same dose of normal saline.

Treatment methods: the control group and DOX group were perfused with 4 mL normal saline every day from the first to the 16th day of the experiment. GLPS (low-dose & high-dose) + DOX group was perfused with GLPS (50 mg/kg & 100 mg/kg) dissolved in 4 ml normal saline for 16 days from the first day of

the experiment. On the 10th day after the last gavage, they were killed and taken.

2.3. Determination of SOD and MDA in Rat Myocardial Tissue

200 mg myocardial tissue was homogenized in a lysate containing 50 mmol/L Tris HCl (pH 7.4), 150 mmol/L NaCl, 5 mmol/L EDTA, 1 mmol/L dithiothreitol, 1% Triton X-100 and 1% protease inhibitor. In the homogenate supernatant, the content of MDA was determined by TBA method and the activity of SOD was determined by WST-1 method.

2.4. The Expression of Related Proteins in Myocardium Was Detected by Western Blot

The protein concentration was determined by BCA protein concentration determination kit, and 50 μg protein was added to SDS-polyacrylamide gel, electrophoretic, transferred to the nitrocellulose membrane, adding the primary antibody (P53, MDM2, HO-1, Nrf2, β-actin), incubating at 4˚C overnight, adding the secondary antibody (diluting the secondary antibody according to 1:500 with diluent) for 1 H at room temperature, and develop color by ECL chemiluminescence method. The ratio of the gray value of the target band to the gray value of the β-actinband was used as the relative expression of each target protein.

2.5. Statistical Analysis

SPSS 25.0 statistical software was used for statistical analysis. All experimental data were expressed as mean ± standard deviation. The four groups' data were compared by one-way ANOVA, and the difference was statistically significant ($p < 0.05$).

3. Results

3.1. Effect of GLPS on the Activities of SOD and MDA in Myocardium Induced by Dox

Compared with the control group, MDA in myocardial tissue of DOX group increased to 42.99 nmol/mg, but SOD expression decreased to 40.95 U/mg. Compared with DOX group, the expression of MDA in myocardium of rats in GLPS low and high dose + DOX group decreased to 8.56 nmol/mg and 18.52 nmol/mg respectively, and the expression of SOD increased to 11.13 U/mg and 23.89 U/mg respectively (**Table 1**, **Figure 1**).

3.2. The Expression Levels of Related Proteins in Each Group Were Detected by Western Blot

The expression of p53 in DOX group was significantly higher than control group, but the expression of MDM2, HO-1 and Nrf2 were significantly lower than control group. The expression of p53 in myocardium of rats in GLPS low and high dose + DOX group were significantly lower than DOX group, and the expression of MDM2, HO-1 and Nrf2 were significantly higher than DOX group (**Table 2**).

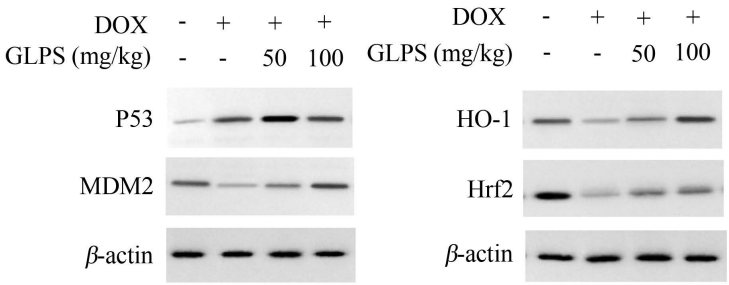

Figure 1. Expression of p53, MDM2, HO-1 and Nrf2 in rat myocardium.

Table 1. Expression of SOD and MDA in myocardial tissue of rats in each group.

	SOD (U/mg)	MDA (nmol/mg)
control	125.6 ± 7.339	8.957 ± 0.489
DOX	84.655 ± 6.281$^{\#}$	51.956 ± 6.474$^{\#}$
GLPS (low) + DOX	95.786 ± 13.188*	43.392 ± 1.173*
GLPS (high) + DOX	108.55 ± 10.432***	33.437 ± 7.045***

GLPS + DOX group compared with DOX group *p < 0.05, **p < 0.01, ***p < 0.001, DOX group compared with control group $^{\#}$p < 0.05.

Table 2. Relative expression of p53, MDM2, HO-1 and Nrf2 in myocardium of rats in each group.

	P53	MDM2	HO-1	Nrf2
control	0.176 ± 0.049	0.971 ± 0.075	1.084 ± 0.104	1.015 ± 0.077
DOX	1.175 ± 0.081$^{\#}$	0.371 ± 0.096$^{\#}$	0.225 ± 0.101$^{\#}$	0.468 ± 0.035
GLPS (low) + DOX	0.798 ± 0.245**	0.532 ± 0.108**	0.395 ± 0.093**	0.531 ± 0.054*
GLPS (high) + DOX	0.441 ± 0.09***	0.77 ± 0.072***	0.612 ± 0.146***	0.7 ± 0.161**

GLPS + DOX group compared with DOX group *p < 0.05, **p < 0.01, ***p < 0.001, DOX group compared with control group $^{\#}$p < 0.05.

4. Discussion

Doxorubicin plays an important role in malignant tumor chemotherapy, but its cardiotoxicity restricts clinical application. Main mechanisms are increased production of reactive oxygen species, mitochondrial dysfunction, DNA damage, and apoptosis [4]. *Ganoderma lucidum* is traditional Chinese medicine, which has lots of biological activities and can be used to prevent and treat a variety of medical diseases. *Ganoderma lucidum* polysaccharide has the functions, such as immune regulation, anti-tumor, liver protection, antioxidant, hypoglycemic, cardiovascular system and neuroprotection. This study confirms that GLPS can reduce the oxidative stress response of myocardial tissue induced by DOX to a certain extent, so as to protect myocardial tissue.

Oxidative stress is an important factor in causing and promoting myocardial injury. MDA and SOD reflect the degree of oxidative stress. MDA can directly

reflect the intensity and rate of lipid peroxidation, and indirectly the degree of tissue damage by free radicals [5]. SOD is an important active component, and the decreasing means the weakening of the body's ability to resist oxidative damage [6]. This study found that DOX abnormally increased MDA and decreased SOD in rat myocardial tissue, suggesting that the myocardial tissue was damaged by free radicals and the antioxidant capacity decreased. GLPS increased the levels of MDA and SOD in myocardial tissue, suggesting that GLPS improved the antioxidant capacity.

Mouse double minute 2 (MDM2) can form a negative feedback loop with p53. The ratio of MDM2/p53 in normal cells is relatively stable. After injury, the expression of MDM2 can be significantly reduced, so as to block the activation of various signal pathways mediated by p53, and finally lead to a variety of pathological injuries such as oxidative stress and inflammation [7]. In this experiment, it is confirmed that DOX can lead to the balance of p53 and MDM2 in myocardial tissue, promote the accumulation of p53 and cause peroxidation in myocardial tissue. This study also finds that GLPS can protect myocardial tissue by increasing the expression of MDM2 and inhibiting p53 activity. Nuclear factor E-2 related factor 2 (Nrf2) is a key regulator to maintain redox homeostasis and cellular antioxidant defense [8], which can regulate the expression of a variety of antioxidants and phase II detoxification enzymes through antioxidant response elements [9]. Heme oxygenase-1 (HO-1) can be used as an antioxidant enzyme to catalyze the degradation of oxidants, so as to prevent inflammation, oxidation and apoptosis. A large number of previous studies have confirmed that increasing HO-1 helps cell resist external stimuli and reduces cell damage caused by oxidative stress [10]. Therefore, Nrf2/HO-1 pathway is considered to be the key mechanism of cell antioxidant defense. We find that GLPS up-regulated Nrf2 nuclear translocation and the expression of downstream target HO-1, indicating that GLPS has antioxidant activity by up-regulating the expression level of Nrf2/HO-1 in myocardial tissue. This confirms that GLPS mediates antioxidant stress by increasing Nrf2 nuclear translocation and HO-1 expression.

In conclusion, GLPS can inhibit oxidative stress and reduce apoptosis by activating p53 negative feedback pathway and Nrf2/HO-1 signal pathway, so as to effectively improve myocardial function and provide a theoretical basis for clinical research.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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