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Study on *Dendrobium officinale* O-acetyl-glucomannan (Dendronan®): Part VI. Protective effects against oxidative stress in immunosuppressed mice

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ABSTRACT

In this study, the antioxidant activities of *Dendrobium officinale* and its two polysaccharide fractions (crude and purified, respectively) in cyclophosphamide induced mice were investigated. All samples examined showed protection for the body from oxidative stress, including increase in activities of catalase (CAT), superoxidase dismutase (SOD), and glutathione peroxidase (GSH-Px), and decrease of malondialdehyde (MDA) content in the serum, thymus and liver. The degree of protection was nearly equally found with the administration of both crude material and purified fractions, indicated that polysaccharide was the main bioactive component, as evidenced by the thymus index and liver index. Together with our previous work in structure study, we suppose that β-(1 → 4)-Man linkage and O-acetyl might be the main causes for the purified polysaccharide (Dendronan®) to exhibit the highest values.

1. Introduction

Reactive oxygen species (ROS) is a collective term of intracellular chemical species that contain oxygen (O_2), and they are reactive to lipids, proteins and DNA (Glasauer & Chandel, 2013). ROS includes both oxygen radicals, like superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet-}$), peroxyl ($RO_2^{\bullet-}$), and hydroperoxyl ($HO_2^{\bullet-}$) radicals; and nonradical oxidizing agents, such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and ozone (O_3), which can be easily transferred into radicals (Bayr, 2005). ROS is involved in various metabolism pathway in cells (Apel & Hirt, 2004), including mitochondrial electron transport, signal transcription, gene expression, enzymatic reactions, and antimicrobial action of neutrophils and macrophages (Bayr, 2005). It is reported that ROS plays a critical role in cell signaling and part of the immune response (Costantini & Møller, 2009; Ray, Huang, & Tsuji, 2012). Certain immune cells, like phagocytes, kill pathogens particularly by releasing pro-oxidant compounds that serve as the starting material for the production of a series of reactive species (Costantini & Møller, 2009; Ray et al., 2012). However, neither excessive amount of ROS nor overdose of antioxidant is beneficial for host health. For examples, over generation of ROS can cause oxidative stress to cellular proteins, nucleic acid and lipids, resulting in membrane damage, DNA damage, and even cell death (Apel & Hirt, 2004; De la Fuente, 2002). Accordingly, the homeostasis of antioxidant/oxidant is of great importance in protecting the hosts from oxidative damage, and at the same time preserving their adequate function as well (De la Fuente, 2002).

It is well acknowledged that many components isolated from original food materials have excellent bioactivities, such as anti-oxidant activity, immunomodulatory activity, and anti-cancer activity. Most of the food derived antioxidants are small molecules, like polyphenol, flavonoid, or short peptides. Polysaccharide, a bioactive macromolecular, is proved to have potential antioxidant activity recently, and has been used as a mild natural antioxidant to protect the body from oxidative stress (Nie & Xie, 2011). *Dendrobium officinale*, an original food material commonly used for making soup or tea, is well known for its unparalleled biological activities, including the abilities of immunostimulation, anti-tumor, anti-cancer, anti-oxidant, anti-inflammatory, alleviating diabetes, and benefiting to gastrointestinal function (Gu et al., 2007;

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Xing et al., 2013). Evidences have revealed that the functional effects of *D. officinale* are closely associated with its polysaccharide (Cai, 2013; Xing et al., 2014).

In our previous study (Xing et al., 2014), two polysaccharide fractions (crude and purified, respectively) were extracted from *D. officinale*. The aim of our study is to understand the protective effects of *D. officinale* and its two polysaccharide fractions (crude extract and purified) against oxidative stress in immunosuppressed mice. The discussion on the structure–bioactivity relationship of the polysaccharide will also be given here.

2. Materials and methods

2.1. Reagents

Cyclophosphamide was purchased from the First Affiliated Hospital of Nanchang University (China). The assay kits for total antioxidant capacity (T-AOC), malondialdehyde (MDA), catalase (CAT), superoxidase dismutase (SOD), and glutathione peroxidase (GSH-Px) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Preparation of DO, p-DOP and c-DOP

D. officinale stem was provided by Jin Jiu Di Bio-technology Ltd. (Lijiang, Yunnan, China). *D. officinale* powder was prepared by ultrafine pulverizing of the dry stem (<80 mesh). An aqueous suspension of *D. officinale* (DO) was produced by constant stirring of the powder in water. The crude polysaccharide (c-DOP) was prepared by water extraction and an ethanol precipitation process. The purified polysaccharide (p-DOP) was prepared by removing the starch from crude fraction (Xing et al., 2014). Our previous studies also indicated that all samples were free of LPS contamination (Cai, 2013). In our series investigations, p-DOP has been identified as O-acetyl-glucomannan (Dendronan®) (Xing et al., 2014, 2015).

2.3. Experimental animals

Male BALB/c mice between 6 and 8 weeks old (weight: 22.0 ± 1.0 g) were purchased from the Vital River (Beijing, China). Animals were allowed to adapt to the new rodent facility for one week before experiment. They were provided with water and mouse chow ad libitum, and housed in the rodent facility at 22 ± 1 °C with a 12-h light–dark cycle for acclimatization. All animal handling procedures involved in current study followed the legislation for the protection of animals used for scientific purpose by the European Commission (EU Directive 2010/63/EU).

2.4. Protocols for immunosuppression induction and treatment

The experimental mice were divided into 12 groups (n = 10), including normal control group (NC), model group (M), three groups for different doses (40, 80, 160 mg/kg body weight) of *D. officinale* powder suspension (DO groups), three groups for different doses of c-DOP (c-DOP groups), three groups for different doses of p-DOP (p-DOP groups) and positive control group (PC group). The immunosuppression mice model was constructed by three days consecutive intraperitoneal injection of 80 mg/kg BW cyclophosphamide (CTX), followed by one injection two weeks later and another one injection three weeks later. On the other days, mice were administrated with saline or polysaccharide samples via gavage in each group. 24 h after the last administration of samples, all the animals were sacrificed for further analysis.

2.5. Biochemical assay

To investigate the antioxidant effects of *D. officinale* and its two polysaccharide fractions on CTX-induced immunosuppression mice, the biochemical parameters, including the total antioxidant capacity (T-AOC), the activity of catalase (CAT), superoxidase dismutase (SOD), and glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) content, were measured for the serum, liver and thymus. The serum was separated from the eyeball blood by centrifugation. Livers and thymus were removed and homogenized with ice-cold physiological saline at 0.1 g/ml wet weight. The homogenates were centrifuged at 3000 ×*g* at 4 °C for 15 min, and the supernatant was collected for further analysis. T-AOC, CAT, SOD, GSH-Px and MAD levels were measured by assay kits. The results of T-AOC, CAT, SOD, and GSH-Px were expressed as active unit per milligram protein, and the results of MDA were expressed as nmol per milligram protein.

2.6. Statistical analysis

The results were expressed as means ± standard deviation (S.D.). A one-way analysis of variance (ANOVA) followed by Dunnett's t-test was employed to evaluate the statistical significance between groups (SPSS 11.0). A value of $P < 0.05$ was regarded as statistical significance.

3. Results

3.1. Effects of oxidative stress caused by cyclophosphamide

Cyclophosphamide (CTX) is a cytotoxic alkylating agents (Kamarzaman, Shaban, & Rahman, 2014) commonly used in scientific research to construct an immunosuppressed mice model. Accumulating studies indicate that cyclophosphamide exposure stimulates the generation of intracellular ROS, suggesting that an oxidative stress occurs during the CTX-induced immunosuppression process (Yuan et al., 2010). In our research, compared with normal group, the liver and thymus indices of model group decreased remarkably after the treatment of CTX (Fig. 1). The results also showed that CTX treatment remarkably down-regulates the total antioxidant capability (T-AOC), the activity of superoxidase dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), and up-regulated the content of malondialdehyde (MDA) in the serum, liver and thymus (Tables 1–3). These findings suggested that CTX induction caused an obvious oxidative stress during this long-lasting immunosuppression process.

3.2. Effects of DO, c-DOP and p-DOP on T-AOC, SOD, CAT, GSH-Px, and MDA levels in the serum

The total antioxidant capability (T-AOC) reflects the capability of non-enzymatic antioxidant defense system (Cui, Yuan, & Zhang, 2010). As shown in Table 1, compared with normal group T-AOC increased dramatically under the treatment of *D. officinale* and its two polysaccharide fractions. This demonstrated that both *D. officinale* and its polysaccharide fractions could alleviate the oxidative damage caused by CTX injection.

It is commonly acknowledged that antioxidant enzymes, such as SOD, CAT and GSH-Px, play critical roles in protecting the host tissue against oxidative stress initiated by superoxide anion (Chen, Hu, & Zheng, 2007). SOD is an important enzyme help to keep the balance of antioxidant/oxidant by scavenging free radicals. CAT is a common reductase distributed in nearly all organisms, catalyzes the decomposition of hydrogen peroxide to water and oxygen. Glutathione (GSH) is also an enzyme that specially catalyzes the decomposition of hydrogen peroxide, resulting in protecting the structure and function of cell membranes. In current study, *D. officinale* and its two polysaccharide fractions were all able to increase the activities of SOD, CAT and GSH-Px in mice serum. Dose dependent manners were clearly observed in p-DOP groups. The results suggested that *D. officinale* and its polysaccharide could reverse the inhibition caused by CTX on the activities of the three antioxidant enzymes.