DOCTORAL THESIS

Immunomodulating effects of natural polysaccharides isolated from astragali radix and dendrobii officinalis caulis

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ABSTRACT

Radix Astragali (the dried root of *Astragalus membranaceous* (Fisch) Bge.) and Dendrobii Officinalis Caulis (the dried stem of *Dendrobium officinale* Kimura et Migo) are two traditional Chinese tonic herbs. They are commonly used in the formula with other Chinese herbs for tonifying Qi, nourishing Yin, and treating various kinds of diseases, such as cancer, diabetes, inflammation, etc. The polysaccharides are considered the majority of the chemical components of decoction boiled from a formula including these two medicinal herbs. The previous study showed that the polysaccharides isolated from Radix Astragali (named RAP) and Dendrobii Officinalis Caulis (named DOP) have various pharmacological activities and most of their activities are closely related to their immunomodulating effects. Nonetheless, the exact mechanism of their immunomodulating effects, especially on macrophages is not known clearly.

In the current study, we have conducted a comprehensive investigation of the bioactive properties and molecular mechanism of immunomodulating activities of DOP and RAP. We aimed to clarify the molecular immunomodulating mechanism of RAP on macrophages and the actual anti-fatigue activity of DOP *in vivo*. Results can be summarized as follows:

RAP itself did not have any cytotoxic effect on mouse mammary carcinoma 4T1 cells, but it significantly enhanced cytotoxicity of the supernatant of RAW264.7cells on 4T1 cells. Furthermore, RAP enhanced the production of NO and cytokines in RAW264.7 cells, and significantly up-regulated gene expressions of TNF-α, IL-6, iNOS. All these bioactivities were blocked by the inhibitor of TLR4 (Toll-like receptor 4), suggesting that TLR4 is a receptor of RAP and mediates its immunomodulating activity. Further analyses demonstrated that RAP rapidly activated TLR4-related MAPKs, including phosphorylated ERK, phosphorylated JNK, and phosphorylated p38, and induced translocation of NF-κB as well as degradation of IκB-α.

In addition, RAP induced higher gene expression of M1 marker, including iNOS, IL-6, TNF-a, CXCL10, compared with those of control group. RAP-induced BMDMs were polarized from M2 to M1 phenotypes. RAP stimulated RAW264.7 cells to express Notch1, Notch2, Jaddge1, Dll1 and SOCS3. Notch signaling pathway played an important role in the RAP-induced polarization of M1 phenotype macrophages. The RAP-induced BMDMs exhibited anti-cancer effect when they were transplanted with 4T1 cells together *in vivo* and it decreased tumor volume and tumor weight.

DOP, the authentication marker of Dendrobii Officinalis Caulis, has immunomodulating activity in macrophage cell line RAW 264.7. DOP enhanced

cell proliferation, TNF-α secretion, and phagocytosis in a dose-dependent manner. It induced the proliferation of lymphocytes alone and with mitogens. For further study the anti-fatigue effect of DOP in vivo, the weight-loaded swimming test was used, because it is an effective method for evaluation of the extent of fatigue. The results indicated that DOP treatment significantly increased the swimming endurance time, body weight, and food intake, compared to the positive control *Rhodiola rosea* extract. Moreover, the weight-loaded swimming test decreased the levels of glycogen in gastrocnemius muscle, SOD, GSH-Px in serum, and increased the levels of LDH, BUN, MDA, CK, TG, and LD in serum. All of these indicators of fatigue were inhibited to a certain extent by both DOP and *Rhodiola rosea* extract, and DOP's effects are stronger. Furthermore, DOP-feeding mice showed significantly increased cell variability of T lymphocytes and B lymphocytes, compared with control mice.

In conclusion, RAP may induce cytokine production of RAW264.7 cells through TLR4-mediated activation of MAPKs and NF-κB. RAP-induced BMDMs were polarized from M2 to M1 phenotypes through Notch signaling pathway. The unique and dominant polysaccharide DOP is proven to be major, active polysaccharide markers of *D. officinale*, and showed stronger anti-fatigue activity than *Rhodiola rosea* extract. As such, DOP has promising potential for pharmaceutical development into anti-fatigue health product.

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