

DOCTORAL THESIS

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

Wei, Wei

Date of Award:
2016

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

ABSTRACT

Radix Astragali (the dried root of *Astragalus membranaceus* (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 RAW264.7 cells 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- κ B as well as degradation of I κ B- α .

In addition, RAP induced higher gene expression of M1 marker, including iNOS, IL-6, TNF- α , 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- κ B. 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.

TABLE OF CONTENTS

DECLARATION.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xiv
CHAPTER 1 INTRODUCTION OF POLYSACCHARIDES ISOLATED FROM RADIX ASTRAGALI AND <i>DENDROBIUM OFFICINALE</i>	1
1.1 General introduction of Radix Astragali	1
1.1.1 Chemical composition of Radix Astragali	3
1.1.2 Studies on bioactivities of Radix Astragali polysaccharides ..	4
1.1.2.1 Immunopotentiating and immunomodulatory activity ..	4
1.1.2.2 Anti-cancer activity	6
1.1.2.3 Antihyperglycemic activity.....	7
1.1.2.4 Anti-inflammatory activity and antioxidant activity	8
1.1.2.5 Antiviral activity	9
1.1.2.6 Other effects	9
1.2 General introduction of <i>Dendrobium officinale</i>	12
1.2.1 Chemical composition of <i>Dendrobium officinale</i>.....	14
1.2.2 Studies on pharmacological activities of polysaccharides from <i>Dendrobium officinale</i>	15
1.3 Objective of this study	17
CHAPTER 2 TLR-4 MAY MEDIATE SIGNALING PATHWAYS OF ASTRAGALUS POLYSACCHARIDE RAP INDUCED CYTOKINE EXPRESSION OF RAW264.7 CELLS	19
2.1 Introduction.....	19
2.2 Materials and methods	22
2.2.1 Materials	22
2.2.2 RAP preparation	23
2.2.3 Cell cultures	25
2.2.4 Cell viability assay	25
2.2.5 Treatment.....	26
2.2.6 RNA extraction and reverse	27
2.2.7 Real-time quantitative PCR.....	27
2.2.8 Measurement of nitric oxide	29
2.2.9 ELISA for quantitative analysis of cytokines	29
2.2.10 Western blot assays of ERK, JNK and p38 MAPKs in	

RAP-induced RAW264.7 cells.....	30
2.2.11 Immunofluorescence staining	30
2.2.12 Statistical analysis	31
2.3 Results	31
2.3.1 RAP enhanced cytotoxicity of supernatants from RAW264.7 cells	31
2.3.2 RAP induced IL-6, NO, and TNF- α production in RAW264.7 cells	34
2.3.3 RAP up-regulated iNOS, TNF- α and IL-6 gene expression	35
2.3.4 TLR4 mediates RAP-induced gene expressions and may participate in the signaling pathways of RAP	35
2.3.5 RAP activated MAPK phosphorylation in RAW264.7 cells....	39
2.3.6 RAP induced I κ B- α degradation and NF- κ B translocation into nucleus.....	42
2.4 Discussion	43
2.5 Conclusion	49
CHAPTER 3 ASTRAGALUS POLYSACCHARIDE RAP INDUCES PHENOTYPE POLARIZATION FROM M2 TO M1 VIA NOTCH SIGNALING PATHWAY	51
3.1 Introduction.....	51
3.2 Materials and methods	55
3.2.1 Materials	55
3.2.1.1 Animals and cells cultures	56
3.2.1.2 Mouse models	58
3.2.2 Analysis of macrophage surface antigen expression by flow cytometry	58
3.2.3 Determination of cytokines by ELISA	59
3.2.4 Determination of NO synthesis.....	59
3.2.5 Cell morphology	59
3.2.6 RNA isolation and RT-PCR	60
3.2.7 Western blot analysis	61
3.2.8 Statistical analysis	63
3.3 Results	64
3.3.1 RAP-stimulated BMDMs decreased tumor volume and tumor weight	64
3.3.2 Morphology of BMDMs induced by RAP	66
3.3.3 Analysis of M1 maker expression on surface of BMDMs	68
3.3.4 Gene expression of Notch signaling pathway induced by RAP	

.....	69
3.3.5 Blocking of Notch signaling pathway results in M1 marker decreased even in the presence of RAP	72
3.4 Discussion	72
3.5 Conclusion	75
CHAPTER 4 IMMUNOMODULATING EFFECTS OF POLYSACCHARIDES ISOLATED FROM <i>DENDROBIUM OFFICINALE</i>	
.....	77
4.1 Introduction	77
4.2 Materials and methods	80
4.2.1 Materials	80
4.2.2 Cell cultures.....	81
4.2.3 Cell viability assay	81
4.2.4 ELISA for quantitative analysis of cytokines	81
4.2.5 Phagocytic assay.....	82
4.2.6 Lymphocyte proliferation assays	83
4.2.7 Data analysis.....	84
4.3 Results	85
4.3.1 Effects of DOP and its two sub-fractions on proliferation of RAW264.7 cells.....	85
4.3.2 Effects of DOP and its two sub-fractions on cytokine production of RAW 264.7 cells.....	86
4.3.3 Phagocytic activities of DOP and its two sub-fractions.....	87
4.3.4 Proliferation of mouse spleen lymphocytes after stimulation with DOP and its two sub-fractions.....	87
4.3.5 Changes of T and B lymphocyte proliferation in synergistical stimulation by DOP and its two sub-fractions with mitogen	89
4.4 Discussion	90
4.5 Conclusion	92
CHAPTER 5 ANTI-FATIGUE EFFECTS OF THE UNIQUE POLYSACCHARIDES MARKER OF <i>DENDROBIUM OFFICINALE</i> ON BALB/C MICE	94
5.1 Introduction	94
5.2 Materials and methods	97
5.2.1 Materials	97
5.2.2 Animals and experimental design	97
5.2.3 Weight-loaded swimming endurance time	99
5.2.4 Biochemical analysis	100

5.2.5	Analysis of tissue glycogen contents	101
5.2.6	Lymphocyte proliferation assays	101
5.2.7	Statistical analysis	102
5.3	Results	104
5.3.1	Effects of DOP and <i>Rhodiola</i> extract on weight-loaded forced swimming endurance time	104
5.3.2	Effects of DOP and <i>Rhodiola</i> extract on body weight and organ indexes	105
5.3.3	Effects of DOP and <i>Rhodiola</i> extract on serum biochemical parameters	109
5.3.4	Effects of DOP and <i>Rhodiola</i> extract on glycogen in liver and gastrocnemius muscle	109
5.3.5	DOP's effect on proliferation of mouse lymphocytes	110
5.4	Discussion	111
5.5	Conclusion	115
CHAPTER 6 SUMMARIES AND PROSPECTS OF THE RESEARCH.....		117
6.1	Summary and conclusion of the research	117
6.2	Prospects of the research	119
6.2.1	Studies on the exact molecular mechanism of RAP stimulating macrophages in vivo and in vitro	119
6.2.2	Further investigate more activity and molecular mechanism of DOP in vivo and in vitro	120
REFERENCES.....		122
CURRICULUM VITAE		146