Bioorganic & Medicinal Chemistry Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Immunoregulatory effect assessment of a novel melanin and its carboxymethyl derivative

Lan Li [a,1], Fang Shi [a,1], Jinglei Li [a], Qianli Huang [b], Can Xu [a], Liuqing Yang [a], Qinghua Yang [b], Farnaz Shaikh [a], Ming Ye [a,*]

[a] Microbial Resources and Application Laboratory, School of Food Science and Engineering, Hefei University of Technology, Hefei 230009, China
[b] School of Biological and Medical Engineering, Hefei University of Technology, Hefei 230009, China

ARTICLE INFO

Article history:
Received 22 November 2016
Revised 16 February 2017
Accepted 17 February 2017
Available online xxxx

Keywords:
Carboxymethylated melanin
Lachnum
Immune regulation effect
Cyclophosphamide

ABSTRACT

A novel melanin with low molecular weight (LIM205, 522 Da) was isolated from the fermentation broth of *Lachnum* sp. and its carboxymethyl derivative (CLIM205) was prepared. The immunoregulatory effects of LIM205 and CLIM205 in immuno-compromised mice induced by cyclophosphamide were investigated. The results demonstrated that both LIM205 and CLIM205 could significantly increase the thymus and spleen indices, specific and nonspecific (including carbon clearance ability) immunity, humoral and cellular immunity of mice. Treatment with LIM205 and CLIM205 could increase activities of SOD, GSH-PX, CAT and decrease content of MDA in the mice. Furthermore, for all animal tests, the immunoregulatory activities of CLIM205 were more prominent than that of LIM205. In conclusion, our findings suggested that the natural products LIM205, as well as its carboxymethyl derivative CLIM205, had significant immunoregulatory activities, which might be a promising source of immunoregulator in healthcare field.

The immune system is the biological defense system which works against the infectious organisms and other invaders. It is comprised by a network of cells, tissues, and organs that work together to protect the body system. In many species, the immune system is classified into some subsystems, such as the innate immune system and the adaptive immune system. Almost all the life processes are related to the immune system, including human embryonic development, occurrence of disease, aging and so on. Therefore, a reasonably balanced immune system is very important for the health of human body.[1–3]

Melanin is a class of phenolic or indole biomolecules. Some water-soluble melanin, such as *Bacillus thuringiensis* melanin,[4] *Inonotus obliquus* melanin,[5] *Castanea mollissima* shells melanin,[6] was reported, but their activities were not well recorded. However, the bioactivity of insoluble natural melanin was reported and got more attention, such as anti-oxidation,[7] immunocompetent,[8] liver-protecting,[9] anti-malarial infection,[10] chelating heavy metals[11] and so on. So melanin is a unique pigment found in all biological kingdoms with myriad functions. *Aspergillus fumigatus* (*A. fumigatus*) melanin could promote immune response against *A. fumigatus* in immunosuppressed mice.[12] Melanin from *Fonsecaea pedrosoi* could induce humoral and cellular responses, protecting the host against chromoblastomycosis.[13] A study demonstrated that *Aspergillus nidulans* melanin had the potentiality as an anti-inflammatory agent and might be used for a new drug candidate with therapeutic utilities.[14] *Lachnum* is a category of saprophytic fungi that can produce a significant amount of melanin under submerged culture conditions which has anti-bacterial,[15] antioxidant, anti-radiation,[16] anti-aging,[17] prevention of alcoholic liver injury[18] and other biological activities. LIM205 was the main homogeneous component of intracellular melanin from *Lachnum* YM205, and insoluble in water or common organic solvents.[19] Both benzothiazole and indole ring were found in the molecular structure of LIM205. The present study was aimed at isolation, identification and immunomodulatory effects evaluations of LIM205 and CLIM205 in hypoimmune mice.

Melanin is usually closely bonded with polysaccharides, protein and lipids, which can be classified into three types, namely umelanin, phaeomelanin and allomelanin. The commonly used method for melanin purification is the so called "alkali extraction and acid precipitation" method, which comprises two steps. The first step is that melanin is dissolved in alkaline solution and the second is that melanin is precipitated in acid solution. The combination of acidolysis and organic solvent extraction was used for purification of melanin in the present study. After removal of the lipid-soluble impurities by extraction, the crude melanin (LIM205) in water

* Corresponding author.
E-mail addresses: yeming123@sina.com, yeming59@163.com (M. Ye).
[1] Lan Li and Fang Shi contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2017.02.046
0960-894X/

Table 1
Effects of LIM205 and CLIM205 for immune organ indices and weight growth in mice.

Groups	Spleen index (mg/g)	Thymus index (mg/g)	Initial weight (mg)	Final weight (mg)
Normal group	5.72 ± 0.19	1.74 ± 0.24	26.50	36.4 ± 2.52
Model control group	4.59 ± 0.28##	0.98 ± 0.14##	27.20	30.3 ± 2.47##
Positive control group	5.46 ± 0.14	1.53 ± 0.23*	26.90	36.2 ± 1.52**
LIM205 (50 mg/kg)	4.61 ± 0.33	1.01 ± 0.31	27.30	32.9 ± 3.03
CLIM205 (50 mg/kg)	4.82 ± 0.61	1.26 ± 0.18	27.20	34.5 ± 2.86
LIM205 (200 mg/kg)	5.13 ± 0.17*	1.37 ± 0.14*	27.50	35.7 ± 1.98**
CLIM205 (200 mg/kg)	5.34 ± 0.11*	1.48 ± 0.11**	28.10	36.1 ± 2.09**

# $P < 0.05$, significantly different from model control group.
* $P < 0.05$, significantly different from Normal group.
** $P < 0.01$, highly significantly different from Normal group.
$P < 0.01$, highly significantly different from model control group.

phase was obtained by evaporation and was isolated on Sephadex G-15 column in succession. The carboxymethylated melanin CLIM205 was prepared according to the method of Wang et al.[20] with slight modifications. The structure characterizations of LIM205 and CLIM205 have been reported in our previous study.[19]

Kidney and spleen are both important immune organs, which play key roles in the production of immune cells and immune responses. Compared with normal group, the mice of model control group were listless, and their fur was loose, messy and dull. In addition, as shown in Table 1, the body weight and immune organs indices were significantly decreased ($p < 0.01$), indicating that immuno-depressed model in mice was successfully established. In comparison with the model control group, the spleen and thymus indices of all LIM205 and CLIM205 groups with different doses were enhanced to a certain extent. The spleen indices of the high-dose LIM205 groups and high-dose CLIM205 groups increased by 11.76% and 16.34% respectively, compared to those of the model control group ($p < 0.05$). The thymus indices of the high-dose LIM205 group and high-dose CLIM205 group increased by 39.8% and 51.04% respectively, compared to those of the model control group ($p < 0.05$). These results indicated that a certain amount of melanin could significantly enhance the thymus and spleen indices of mice, thus facilitating the recovery from thymus and spleen damage. In addition, the thymus and spleen indices of CLIM205 group were higher than those of LIM205 in the same doses and the high-dose CLIM205 group showed the same trend of increase as the positive control groups.

Nonspecific immunity serves as the basis of specific immunity. Foreign substances can be quickly recognized and cleared by macrophages when they invaded into the body system. Carbon clearance index is used as the main reflection of macrophage elimination ability against foreign substances. Compared with normal group, carbon clearance index of model control group fell down to 38.95%. Compared with the model control group, the improved carbon clearance index was observed in all melanin treated groups. High dose CLIM205 group had similar pharmacodynamics as positive control group and was slightly more effective than LIM205 with the same doses, indicating that both LIM205 and CLIM205 could improve nonspecific phagocytosis of immunocompromised mice and CLIM205 was a little more effective than LIM205.

Macrophages are important immune cells in body's immune system and phagocytic index is another important indicator to reflect the nonspecific immune functions of organism.[21] Macrophages induced by some factors can be converted into active macrophages with a strong ability of anti-microbial infection and killing tumor cells.[22] In Table 2, peritoneal macrophages rate and phagocytic index in model control group were 21.33 ± 0.98 and 0.50 ± 0.03, respectively, which were significantly lower than normal group level ($p < 0.01$), suggesting that the model control group had low nonspecific immune function.

Each drug group could increase peritoneal macrophage phagocytic percentage and phagocytic index in cyclophosphamide-induced immunosuppressed mice. High-dose CLIM205 group was close to positive drug group but was more effective than LIM205 group in the same dose.

Hemolysin is an important index for evaluating animal humoral immunity function. When the mice were injected with SRBC (antigen), B lymphocytes in body would secrete an antibody, which was an indicator of humoral immunity.[23] Compared with the normal group, HC_{50} of the cyclophosphamide model group was reduced by 12.2%. However, compared with the model control group, high dose LIM205 group and CLIM205 could significantly increase the HC_{50} values of mice ($p < 0.05$), and the high dose CLIM205 group had similar HC_{50} as the positive drug group. It revealed that *Lachnum* melanin modified with carboxymethyl moiety could promote B lymphocytes in proliferation, differentiation and secretion of more anti-SRBC antibodies.

After being injected with SRBC for several days, B lymphocytes in model mice would produce SRBC antibodies. With the participation of alexin, SRBC would be lysed. Therefore, the level of antibody

Table 2
Effects of LIM205 and CLIM205 for nonspecific immunity in mice.

Groups	Carbon clearance index	Macrophage phagocytosis	
		Phagocytosis rate (%)	Phagocytosis index
Normal group	4.39 ± 0.27	28.13 ± 1.20	1.03 ± 0.07
Model control group	2.68 ± 0.30##	21.33 ± 0.98##	0.50 ± 0.03##
Positive control group	3.87 ± 0.32*	26.75 ± 0.62**	0.89 ± 0.03**
LIM205 (50 mg/kg)	2.95 ± 0.15	22.14 ± 1.08*	0.53 ± 0.05
CLIM205 (50 mg/kg)	3.21 ± 0.35	24.09 ± 0.83*	0.60 ± 0.11*
LIM205 (200 mg/kg)	3.54 ± 0.23*	26.31 ± 1.12**	0.79 ± 0.12*
CLIM205 (200 mg/kg)	3.76 ± 0.33*	27.01 ± 1.13**	0.83 ± 0.13*

# $P < 0.05$, significantly different from model control group.
* $P < 0.05$, significantly different from Normal group.
** $P < 0.01$, highly significantly different from Normal group.
$P < 0.01$, highly significantly different from model control group.

Please cite this article in press as: Li L., et al. Bioorg. Med. Chem. Lett. (2017), http://dx.doi.org/10.1016/j.bmcl.2017.02.046