Different level of tumor necrosis factor-α expression after administration of silk sericin fraction in RAW264.7 cells

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Abstract

Tumor necrosis factor-α (TNFα) is a representative marker for inflammation. Silk sericin is known as mild TNFα inducer. The purpose of this study was to compare the level of TNFα among different fractions of silk sericin. Silk sericin was extracted from cocoon and separated it by molecular weight. Each fraction was applied to RAW264.7 cells. The level of TNFα was evaluated by western blot and ELISA assay. In results, the level of TNFα was increased as time-dependent manner. Higher molecular weight fraction of sericin induced higher amount of TNFα than lower molecular weight fraction. In conclusion, different molecular weight fraction of sericin induced TNFα differently.

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Introduction

The cocoon of silkworm is mainly composed of silk fibroin and silk sericin (Park and Um, 2018). Silk sericin is removed during degumming process and it is considered as industrial waste (Park and Um, 2016). Recently, beneficial effect of silk sericin has been clarified (Gholipourmalekabadi et al., 2020; Ahsan et al., 2018). Accordingly, silk sericin has been widely used for medical and cosmetic purpose. Silk sericin can be produced by degumming process (Park and Um, 2016, 2018). There are different types of degumming process (Bae et al., 2016). Biological effect of protein is dependent on its conformation and molecular weight. The conformation and molecular weight of silk protein should be different to its degumming process (Bae et al., 2016). There have been several reports about the biological effect of silk sericin according to its degumming process and biological effect of silk sericin is different to its degumming process (Nuchadomrong et al., 2019; Zhang et al., 2006). However, the biological effect of silk sericin according to its molecular weight has not been clarified when it is extracted by the same degumming process.

Sericin is glue-like protein. There are 3 types of silk sericin. Among them, sericin 1 and sericin 3 are main component of silkworm cocoon (Kaur et al., 2013; Zhang et al., 2015). The composition of sericin 1 and sericin 3 are different to layers. Sericin 1 is abundant in the outer layer (Kaur et al., 2013; Zhang et al., 2015) and sericin 3 is lower in the middle layer (Zhang et al., 2015). Main amino acid of silk sericin is serine. Silk sericin is mostly hydrophilic protein. The molecular weight of sericin 1 and sericin 3 from Bombyx mori is approximately 120 kDa according to National Center for Biotechnology Information.

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and 10 mg/mL of total sericin, filtered, or unfiltered sericin. The media volume and seeding number were in accord to standard protocol (working volume: 1.8 mL, seeding density: 0.3 x 10⁶). After 2, 8, or 24 h of culture, the cells were collected. Cells in the control culture were treated with a volume of solvent equivalent to that required for sericin.

Western blot and enzyme-linked immunosorbent assay (ELISA)

Proteins were collected and mixed with a sodium dodecyl sulfate buffer. After heat denaturation, they were electrophoresed on 10% polyacrylamide gels. The gels were transferred to polyvinylidene difluoride membranes. After blocking, the membranes were probed with primary antibodies for TNF-α (dilution ratio = 1:500). Blots were imaged and quantified using a ChemiDoc XRS system (Bio-Rad Laboratories).

ELISA for TNF-α was performed for RAW264.7 cells. Silk sericin was applied to RAW264.7 cells at concentrations of 1, 5, and 10 μg/mL, and the supernatant was collected. ELISA was conducted using a commercially available kit (Cat#: ab208348, Abcam, Cambridge, UK) and the detailed protocol supplied by the manufacturer.

Results

The results of western blot demonstrated that the application of silk sericin increased the expression level of TNF-α as time- and dose-dependent manner (Fig. 1). When compared between filtered fractions, high molecular fraction of silk sericin (M.W. > 30kDa) showed higher level of TNF-α expression compared to low molecular fraction (M.W. < 30 kDa).

The results of ELISA were also in accord to those of western blot (Fig. 2). The application of silk sericin showed increased level of TNF-α expression as dose- and time-dependent manner. The application of 10 μg/mL total silk sericin induced 275.82 ± 9.59 pg/mL of TNF-α at 24h after administration (Fig. 2a). In case of 1 and 5 μg/mL total silk sericin induced 141.05 ± 8.49 pg/mL and 175.74 ± 14.81 pg/mL of TNF-α at 24h after administration, respectively. The difference among groups was statistically significant (P<0.05). The application of 10 μg/mL high molecular fraction silk sericin (M.W. > 30 kDa) induced 197.87 ± 9.64 pg/mL of TNF-α at 24h after administration (Fig. 2b).
These immune reactions are mainly mediated by monocytes/macrophages. Macrophages have pattern recognizing receptors. TLR is representative pattern recognizing receptor (Dowling and Dellacasagrande, 2016). When foreign materials are bound to TLR, conformation of TLR is changed and signal is generated subsequently (Dowling and Dellacasagrande, 2016). The signal transmitted from TLR activates nuclear factor-κB (NF-κB) pathway (Andrade-Oliveira et al., 2019; Padron et al., 2020). As a result, the transcription
of TNFα is increased and TNFα protein level is also increased (Thoma and Lightfoot, 2018). Accordingly, TNFα expression level induced by silk sericin administration might be dependent of signal intensity generated by TLR activation. The intensity of TLR activation is dependent of foreign material conformation, amount, and charge. Therefore, high molecular weight fraction of silk sericin (M.W. > 30 kDa) might activate TLR stronger than low molecular weight fraction of silk sericin (M.W. < 30 kDa).

The silk sericin released from silkworm cocoon is different to layers (Jo et al., 2017). The middle layer of silkworm cocoon shows lowest amount of sericin release and the TNFα expression level is also lowest in the middle layer (Jo et al., 2017). The different profile of released proteins from each silkworm cocoon layer results in different types of cellular response (Kim et al., 2018). Though high molecular weight fraction of silk sericin (M.W. > 30 kDa) increased TNFα expression level more than low molecular weight fraction of silk sericin (M.W. < 30 kDa), the decision between them would be dependent on intended purpose. In terms of tissue engineering, both M1 and M2 type polarizing agent are important for uneventful wound healing (Kim, 2020). In addition, TNFα expression level after silk sericin administration in this study was only picogram level (Fig. 2). Accordingly, the method for the extraction of silk sericin from silkworm cocoon should be tailored to its purpose.

The limitations of this study were as follows. First, there are many kinds of degumming process. However, only sonication in body temperature was used in this study. The effect of other degumming process should be studied in following studies. Second, TLR was mentioned as main target receptor for silk sericin. However, the expression level of TLR or its downstream signaling pathway had not been studied in this manuscript. Though TLR is main receptor for pattern recognition of foreign material (Dowling and Dellacasagrande, 2016), the association between TLR and silk sericin should be clarified. Third, other proteins except for silk sericin are found in the silkworm cocoon (Zhang et al., 2015). Most of these proteins have low molecular weight (<30 kDa) (Zhang et al., 2015). The biological effect of these proteins also should be clarified. Fourth, sericin is hydrophilic, however, is not water soluble in its non-fragmented form (Park and Um, 2018). Sericin molecules and swollen or aggregated particles are dispersed in solution. Accordingly, it was very difficult to divide sericin fragment exactly by the filter on the base of molecular weight. For the precise extraction of sericin according to its molecular weight, more precise instrument will be required.

Conclusion

In this study, silk sericin induced different level of TNFα protein expression according to its molecular weight in RAW264.7 cells. Higher molecular weight fraction of silk sericin (M.W. > 30 kDa) induced significantly higher level of TNFα expression compared to lower molecular weight fraction (M.W. < 30 kDa).

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References

Bone regeneration is associated with the concentration of tumour necrosis factor-α induced by sericin released from a silk mat. Sci Rep 7, 15589.


