SHORT COMMUNICATION

Nucleosides and amino acids, isolated from Cordyceps sinensis, protected against cyclophosphamide-induced myelosuppression in mice

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ABSTRACT

The material basis of Cordyceps sinensis (Berk.) Sacc has not yet been well understood and natural C. sinensis resources are very rare. The present study aimed to clarify the substance basis and compare the protective effect of natural and artificially-cultivated C. sinensis against cyclophosphamide (CTX)-induced myelosuppression. Both natural and artificially-cultivated C. sinensis effectively improved CTX-induced decrease of peripheral blood counts and hemopoietic growth factors, pathological changes, and apoptosis of bone marrow. Importantly, artificially-cultivated C. sinensis showed similar capacity compared with natural C. sinensis. Uridine (1), adenosine (2), L-pyroglutamic acid (3), lysinonorleucine (4), 1,3,5-trimethoxybenzene (5), D-mannitol (6), L-pyroglutamic acid methyl ester (7), tryptophan (8), and phenylalanine (9) were isolated from bioactivity-guided fraction and identified to attenuate CTX-induced myelosuppression in mice. In conclusions, nucleosides and amino acids represented the effective chemical components in C. sinensis. Artificial cultivation can be used as an effective substitute for natural C. sinensis.

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1. Introduction

*Cordyceps sinensis* (Berk.) Sacc. is a worm in winter but a grass in summer. For centuries, *C. sinensis* has been used as an immunomodulator in China (Qian et al. 2012; Kang et al. 2015). Due to its extreme host range specificity, *C. sinensis* grows slowly and natural resources are scarce. The overexploitation has made *C. sinensis* on the brink of extinction. Recently, artificially-cultivated *C. sinensis* has been successfully developed in China (Li, Liu, et al. 2019). However, the active ingredients of cultivated *C. sinensis* has not been fully understood, which needs to be further characterised. In present study, the potential effect on CTX-induced myelosuppression was evaluated in mice after treatment of natural and artificially-cultivated *C. sinensis*. The active components were identified. The results would provide useful information to understand the effect of *C. sinensis* for improving hematopoietic function and promote the development process of the cultivated substitute to protect the *C. sinensis* resources on the verge of extinction.

2. Results and discussion

2.1. Natural and artificially-cultivated *C. sinensis* exhibited virtually identical protective effect against CTX-induced myelosuppression in mice

To evaluate the potential effect of natural and artificially-cultivated *C. sinensis* on CTX-induced myelosuppression, their chemical compositions were firstly analyzed by HPLC, which showed similar chemical composition (Supplementary Figure 1A). Both natural and cultivated strains, significantly improved CTX-induced myelosuppression in mice, as evidenced by increased levels of immune organ indexes, peripheral blood counts, EPO, G-CSF and TPO (Supplementary Figures 2–4). Moreover, *C. sinensis* increased bone marrow cell number, improved femoral bone morphology and inhibited cell apoptosis (Supplementary Figures 5–7). These findings suggested the chemoprotective effects of natural and artificially-cultivated *C. sinensis* were identical, which means artificial cultivation can be used as an effective substitute for natural *C. sinensis* regarding the effect on CTX-induced myelosuppression.
2.2. Water extracts of C. sinensis contribute to the protective effect against CTX-induced myelosuppression in mice

In order to elucidate the active components, C. sinensis was extracted by different extract solvents and only water extract increased WBC counts and LYM% level (Supplementary Table 1). These results suggested that the active components of C. sinensis responsible for the leukogenic effects might be water-soluble substances with higher polarity. Then, the exact compounds in ethanol extract and water extract were systematically isolated using preparative HPLC and silica gel column chromatography. Finally, compounds 1-9 were obtained and their chemical structures were identified by 1H and 13C NMR data (Supplementary Figure 9–26), namely uridine (1) (Kwon et al. 2003), adenosine (2) (Shao et al. 2014), L-pyroglutamic acid (3) (Gao et al. 2001), lysino-norleucine (4) (van den Nieuwendijk et al. 1999), 1,3,5-trimethoxybenzene (5) (Xie et al. 2013), D-mannitol (6) (Yang et al. 2005), L-pyroglutamic acid methyl ester (7) (He et al. 2014), tryptophan (8) (Yuan et al. 2009), phenylalanine (9) (Wang et al. 2017) (Supplementary Figure 1B). Among them, the contents of adenosine, uridine, mannitol, tryptophan, phenylalanine and pyroglutamic acid in natural and artificially-cultivated C. sinensis were determined by LC-MS/MS (Supplementary Figure 1C; Supplementary Table 2). Natural and artificially-cultivated C. sinensis contained similar contents of these compounds with variations of 0.48–1.78 (contents ratio of artificially-cultivated C. sinensis to natural C. sinensis) (Supplementary Table 3). Actually, the main nutrients including soluble proteins, nucleosides, nucleotides, and adenosine between the natural and artificially-cultivated samples were virtually identical, while the toxic heavy metal levels were significantly lower in artificially-cultivated C. sinensis (Lee et al. 2015; Li, Han, et al. 2019; Zhou et al. 2019; Guo et al. 2020). Then, the effects of adenosine, uridine, mannitol, tryptophan, phenylalanine and pyroglutamic acid were directly evaluated in CTX-induced myelosuppression mice. First, all six compounds abolished the body weight loss induced by CTX but failed to ameliorate the decrease of indexes of thymus and spleen (Supplementary Figure 27). Furthermore, six compounds displayed varied effects on hematopoietic function of bone marrow in CTX-induced mice. It should be noted that the protective potential of a single compound verified in present study was poorer than that of C. sinensis. These results suggested that these compounds ameliorated CTX-induced myelosuppression to different degrees and the leukogenic effect of C. sinensis could be a synergistic action of multiple components, like adenosine, uridine, mannitol, tryptophan, phenylalanine and pyroglutamic acid.

3. Conclusion

Both natural and artificially-cultivated C. sinensis exhibited comparable pharmacological activity against CTX-induced myelosuppression in mice. Nucleosides and amino acids represented the effective chemical components in C. sinensis. The findings suggested that artificial cultivation can be used as an effective substitute for natural C. sinensis regarding the effect on CTX-induced myelosuppression.
Disclosure statement

No potential conflict of interest was reported by the authors.

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