Editorial Commentary

Coupled biosynthesis of cordycepin and pentostatin in *Cordyceps militaris*: implications for fungal biology and medicinal natural products

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Cordycepin, or 3'-deoxyadenosine, is a metabolite produced by the insect-pathogenic fungus *Cordyceps militaris (C. militaris)* and is under intense investigation as a potential lead compound for cancer and inflammatory conditions. Cordycepin was originally extracted by Cunningham *et al.* (1) from a culture filtrate of a *C. militaris* culture that was grown from conidia. Subsequently, cordycepin has also been reported to be produced by *Ophiocordyceps sinensis* (2), a species historically used as a traditional medicine and health food, primarily in China and the wider Far East (3). Cultivated *C. militaris* is now widely in use as less expensive substitute. In addition, these fungi are also globally gaining a market as natural food supplements, with 30–50 products claiming to contain *Cordyceps* available in the UK and the USA.

A large body of literature (too much to cite comprehensively here) indicates that cordycepin indeed has biological activities that indicate it may have pharmaceutical potential. In tissue culture, anti-inflammatory properties and anti-tumour effects are especially well established (4-9). In addition, it has been shown to be effective in numerous animal models of disease, including models for osteoarthritis, inflammatory lung disease, cerebral ischaemia, kidney failure and cancer (9-17). Our own work on pain in models of osteoarthritis suggest that cordycepin acts as a novel type of anti-inflammatory painkiller (11). To our knowledge, no conclusive data from clinical trials with cordycepin have been published. However, even if only one of the many reported effects on animal disease models can be replicated in people, this could become a very important new natural product-derived medicine.

Cordycepin is known to be unstable in animals due to deamination by adenosine deaminases. Much of the efforts towards bringing cordycepin to the clinic have been focussed on chemical modifications, formulations and co-administration with adenosine deaminase inhibitors such as pentostatin (18-22). Notably, the majority of commercially available cordycepin products, and certainly all the most affordable preparations, are still isolated from cultivated fungi.

It was therefore of great interest that we read the recent paper by Xia *et al.* [2017] (23). The authors showed that in *C. militaris* the production of cordycepin is coupled with the production of the adenosine deaminase inhibitor pentostatin; with genes essential for their synthesis in adjacent loci, *cns1*, *cns2*, and *cns3* (23). Functional verification of the genes *cns1* and *cns2* for cordycepin production was performed by generating *Aspergillus nidulans* knockout mutants and heterologous gene expression in *Metarhizium robertsii* and *Saccharomyces cerevisiae*. Similarly, heterologous expression of *cns3* in *M. robertsii* and *Cordyceps bassiana* confirmed the role of Cns3 for pentostatin production. Yeast two-hybrid and co-localisation-based evidence for Cns1 and Cns2 protein interaction was also provided (23). This work is certainly important for the optimisation of *C. militaris* cordycepin production strains. In addition, there are wider implications on the ecology of secondary

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metabolites and their potential applications.

Surprisingly, Xia *et al.* failed to detect cordycepin production in species closely related to *C. militaris* such as *C. bassiana*, *C. confragosa*, *C. takaomontana*, *Ophiocordyceps sinensis*, *Isaria fumosorosea*, *Metarhizium robertsii*, and *M. rileyi*, which is in agreement with the lack of homologous genes for its biosynthesis in these species (23). In the case of *O. sinensis*, this is particularly puzzling, as it contradicts previous studies (2). If *O. sinensis* indeed produces cordycepin under certain conditions, a non-conserved pathway involving different enzymes may be used. Alternatively, fungi collected from the wild may be associated with other cordycepin-producing organisms. This speculation is supported by the fact that, when detected, the amount of cordycepin found in *O. sinensis* is low compared to the levels in *C. militaris* (2). Interestingly, cordycepin biosynthesis genes similar to those from *C. militaris* were found in the phylogenetically distant species *Aspergillus nidulans* (a eurotiomycete, in a different ascomycete class) and *Acremonium chrysogenum*. We therefore consider it possible that this fascinating "protector-protégé" system for the production of pentostatin and cordycepin was acquired by gene transfer between different species. Horizontal gene transfer has been widely proposed to occur in fungi based on genome structure, although it has not been observed directly (24).

The co-production of cordycepin and pentostatin in *C. militaris* is likely the result of the evolutionary pressures on this insect-infecting fungus, with pentostatin keeping cordycepin in its active form. A probable, but so far unconfirmed, hypothesis is that cordycepin represses the immune system of the insect host, which lacks adaptive immunity. Indeed, cordycepin has been attributed as the proximate cause of insect host death following colonisation of the insect by *C. militaris* (25). Therefore, the effect of cordycepin, pentostatin, and other secondary metabolites on insect immune systems and fungal infection are worth investigating. This could lead to biological control applications for targeting insect pests. Although *O. sinensis* may not produce cordycepin, it is subject to similar evolutionary pressures as *C. militaris* and therefore possibly produces different compounds with similar effects on insect and mammalian immune systems. Therefore, if it can be confirmed that secondary metabolites from insect-infecting fungi target the insect immune system, this will suggest that more such useful compounds may be found in this ecological niche.

Beyond the impact of this paper on cordycepin production and the biology of insect-infecting fungi, the study by Xia *et al.* also has implications for how we test biological activity of natural compounds. If we take into account that the evolution of natural compounds is likely to have led to synergistic mixtures, there appears to be a case for initially testing mixtures, rather than pure compounds, as activity may be lost by purification of single compounds. Natural compounds, their synthesis and their activities are likely to provide a rich source for exciting discoveries for many years to come.

Acknowledgments

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Cunningham KG, Manson W, Spring FS, et al. Cordycepin, a metabolic product isolated from cultures of Cordyceps militaris (Linn.) Link. Nature 1950;166:949.
2. Huang LF, Liang YZ, Guo FQ, et al. Simultaneous separation and determination of active components in Cordyceps sinensis and Cordyceps militaris by LC/ESI-MS. J Pharm Biomed Anal 2003;33:1155-62.
3. Winkler D. Cordyceps sinensis: a precious parasitic fungus infecting Tibet. Field Mycol 2010;11:60-7.
4. Kim HG, Shrestha B, Lim SY, et al. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-kappaB through Akt and p38 inhibition in RAW 264.7 macrophage cells. Eur J Pharmacol 2006;545:192-9.
5. Ren Z, Cui J, Huo Z, et al. Cordycepin suppresses TNF-alpha-induced NF-kappaB activation by reducing p65 transcriptional activity, inhibiting IkappaBalpha phosphorylation, and blocking IKKgamma ubiquitination. Int Immunopharmacol 2012;14:698-703.
6. Choi YH, Kim GY, Lee HH. Anti-inflammatory effects of cordycepin in lipopolysaccharide-stimulated RAW 264.7 macrophages through Toll-like receptor 4-mediated suppression of mitogen-activated protein kinases and NF-kappaB signaling pathways. Drug Des Devel Ther 2014;8:1941-53.
7. Kondrashov A, Meijer HA, Barthet-Barateig A, et al.

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Inhibition of polyadenylation reduces inflammatory gene induction. RNA 2012;18:2236-50.
8. Thomadaki H, Tsiapalis CM, Scorilas A. The effect of the polyadenylation inhibitor cordycepin on human Molt-4 and Daudi leukaemia and lymphoma cell lines. Cancer Chemother Pharmacol 2008;61:703-11.
9. Wang XA, Xiang SS, Li HF, et al. Cordycepin induces S phase arrest and apoptosis in human gallbladder cancer cells. Molecules 2014;19:11350-65.
10. Kim H, Naura AS, Errami Y, et al. Cordycepin blocks lung injury-associated inflammation and promotes BRCA1-deficient breast cancer cell killing by effectively inhibiting PARP. Mol Med 2011;17:893-900.
11. Ashraf S, Radhi M, Gowler P, et al. The polyadenylation inhibitor cordycepin reduces pain, inflammation and joint pathology in rodent models of osteoarthritis. Sci Rep 2019;9:4696.
12. Tianzhu Z, Shihai Y, Juan D. The Effects of Cordycepin on Ovalbumin-Induced Allergic Inflammation by Strengthening Treg Response and Suppressing Th17 Responses in Ovalbumin-Sensitized Mice. Inflammation 2015;38:1036-43.
13. Yang X, Li Y, He Y, et al. Cordycepin alleviates airway hyperreactivity in a murine model of asthma by attenuating the inflammatory process. Int Immunopharmacol 2015;26:401-8.
14. Cheng Z, He W, Zhou X, et al. Cordycepin protects against cerebral ischemia/reperfusion injury in vivo and in vitro. Eur J Pharmacol 2011;664:20-8.
15. Kitamura M, Kato H, Saito Y, et al. Aberrant, differential and bidirectional regulation of the unfolded protein response towards cell survival by 3'-deoxyadenosine. Cell Death Differ 2011;18:1876-88.
16. Nakamura K, Konoha K, Yoshikawa N, et al. Effect of cordycepin (3'-deoxyadenosine) on hematogenic lung metastatic model mice. In Vivo 2005;19:137-41.
17. Zhang P, Huang C, Fu C, et al. Cordycepin (3'-deoxyadenosine) suppressed HMGA2, Twist1 and ZEB1-dependent melanoma invasion and metastasis by targeting miR-33b. Oncotarget 2015;6:9834-53.
18. Rodman LE, Farnell DR, Coyne JM, et al. Toxicity of cordycepin in combination with the adenosine deaminase inhibitor 2'-deoxycoformycin in beagle dogs. Toxicol Appl Pharmacol 1997;147:39-45.
19. Dalla Rosa L, Da Silva AS, Gressler LT, et al. Cordycepin (3'-deoxyadenosine) pentostatin (deoxycoformycin) combination treatment of mice experimentally infected with Trypanosoma evansi. Parasitology 2013;140:663-71.
20. Vodnala SK, Lundback T, Yeheskieli E, et al. Structure-activity relationships of synthetic cordycepin analogues as experimental therapeutics for African trypanosomiasis. J Med Chem 2013;56:9861-73.
21. Zhao Z, Song G, Tian H, et al. Triacetyl-3-hydroxyphenyladenosine, a derivative of cordycepin, attenuates atherosclerosis in apolipoprotein E-knockout mice. Exp Biol Med (Maywood) 2012;237:1262-72.
22. Aramwit P, Porasuphatana S, Srichana T, et al. Toxicity evaluation of cordycepin and its delivery system for sustained in vitro anti-lung cancer activity. Nanoscale Res Lett 2015;10:152.
23. Xia Y, Luo F, Shang Y, et al. Fungal cordycepin biosynthesis is coupled with the production of the safeguard molecule pentostatin. Cell Chem Biol 2017;24:1479-89.e4.
24. Rosewich UL, Kistler HC. Role of horizontal gene transfer in the evolution of fungi. Annu Rev Phytopathol 2000;38:325-63.
25. Kim JR, Yeon SH, Kim HS, et al. Larvicidal activity against Plutella xylostela of cordycepin from the fruiting body of Cordyceps militaris. Pest Manag Sci 2002;58:713-7.

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