Minireview

Cordycepin: A bioactive metabolite with therapeutic potential

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Abstract

Cytotoxic nucleoside analogues were the first chemotherapeutic agents for cancer treatment. Cordycepin, an active ingredient of the insect fungus Cordyceps militaris, is a category of compounds that exhibit significant therapeutic potential. Cordycepin has many intracellular targets, including nucleic acid (DNA/RNA), apoptosis and cell cycle, etc. Investigations of the mechanism of anti-cancer drugs have yielded important information for the design of novel drug targets in order to enhance anti-tumor activity with less toxicity to patients. This extensive review covers various molecular aspects of cordycepin interactions with its recognized cellular targets and proposes the development of novel therapeutic strategies for cancer treatment.

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Introduction

The biometabolite cordycepin was first isolated from the fermented broth of the medicinal mushroom Cordyceps militaris (Cunningham et al., 1950), which is an entomopathogenic fungus that grows parasitically on lepidopteron larvae and insect pupae. The genus Cordyceps is well-known in traditional Chinese medicine and exhibits a variety of clinical health effects including immunomodulatory, anticancer, antioxidant, anti-inflammatory and anti-microbial activities (Tuli et al., 2013). This medicinal mushroom has been found naturally at an altitude of approximately 14,000 ft in the Himalayan lands including Nepal, China, Tibet and India (Meena et al., 2010). This mushroom is also known as winter worm summer grass in China where it is known locally as keeda ghas. Because of the specific distribution and potent medicinal value of the fungus, the market price of Cordyceps species is approximately US $12,000 kg−1 (Paterson, 2008). In addition to cordycepin, Cordyceps have been known to produce a variety of other pharmacologically active compounds, such as adenosine, exo-polysaccharides and sterols (Huang et al., 2009; Xie et al., 2010; Yan et al., 2008; Zhong et al., 2009).

Cordycepin is a type of nucleoside analogue that is structurally similar to adenosine, except that it lacks a 3′ hydroxyl group, which increases its potency, and it is known to interfere with a number of
biochemical and molecular processes, such as purine biosynthesis (Overgaard, 1964; Rottman and Guarino, 1964), DNA/RNA synthesis (Holbein et al., 2009) and mTOR (mammalian target of rapamycin) signaling transduction (Wong et al., 2010). In addition to these classical activities, other molecular targets of cordycepin include apoptosis, cell cycle arrest, anti-metastasis, inhibition of platelets aggregation and inflammation mediators’ pathways (Fig. 1). However, the cure of diseases such as cancer still remains elusive despite the availability of a variety of chemotherapeutics agents that exhibit sophisticated mechanisms of action. Therefore, in-depth knowledge of nucleoside analogues, such as cordycepin, is essential not only to understand the biology behind cancer but also to develop novel therapeutic strategies.

This review describes the variety of molecular mechanisms that mediate the pharmacological effects of cordycepin and the use and biological applications of cordycepin-based derivatives.

**Chemistry of cordycepin**

Cordycepin, or 3′-deoxyadenosine (9-(3-deoxy-β-D-ribofuranosyl)adenine), is an adenosine analogue (Fig. 2), molecular formula C_{10}H_{13}N_{5}O_{3}, molecular weight 251.24, alkaline, needle-like or flaky crystal, melting point 228 °C–231 °C, with a maximum absorption wavelength of 259.0 nm. The structure of cordycepin comprises a purine (adenine) nucleoside molecule attached to a ribose sugar (ribofuranose) moiety via a β-N9-glycosidic bond. The chemical synthesis of cordycepin is achieved mainly through the replacement of the OH group at the 3′ position in the ribofuranosyl moiety with H, generating a deoxy analogue of adenosine. Cordycepin can behave as a bidentate ligand because of the 5 nitrogen and 3 oxygen atoms through which it binds with the transition metals.

**Inhibition of purine biosynthesis, DNA/RNA biosynthesis, and mTOR signaling pathway**

Once inside the cell, cordycepin is converted into 5′ mono-, di- and tri-phosphates that inhibit the activity of enzymes, such as ribose-phosphate pyrophosphokinase and 5-phosphoribosyl-1-pyrophosphate amidotransferase, which are used in the de novo biosynthesis of purines (Klenow, 1963; Overgaard, 1964; Rottman and Guarino, 1964). Because of its similarity with adenosine, cordycepin can participate in various molecular processes in cells, such as the synthesis of DNA and/or RNA. During the process of transcription (RNA synthesis), a number of enzymes cannot distinguish between an adenosine and cordycepin, which leads to the incorporation of 3′-deoxyadenosine or cordycepin, in place of the normal nucleoside, preventing further incorporation of nitrogenous bases (A, U, G, and C), leading to the premature termination of transcription (Chen et al., 2008; Holbein et al., 2009). Wong et al. (2010) reported the role of cordycepin in the shortening of the poly-A tail of mRNA, inhibition of cell attachment and reduction in focal adhesion. Furthermore, the study suggested that cordycepin may shut down the mTOR signaling pathway by activating the AMP activated kinase (AMPK) through an unknown mechanism, which leads to the inhibition of translation, cell proliferation and growth.

**Induction of apoptosis and cell cycle regulation**

Apoptosis, also referred to as programmed cell death, is characterized mainly by a series of distinct changes in cell morphology, such as blebbing, loss of cell attachment, cytoplasmic contraction, DNA fragmentation and other biochemical changes including the activation of caspases through extrinsic and/or intrinsic mitochondrial pathways. Past research has demonstrated the role of cordycepin in the induction of apoptosis through regulating the expression of various proteins. This process involves programmed cell death, for example, the Bcl-2 family of proteins has both anti-apoptotic and pro-apoptotic members. Cordycepin has also been known to induce apoptosis in human colorectal cancer cell lines (SW480 and SW620) by enhancing the protein expression levels of JNK, p38 kinase and Bcl-2 pro-apoptotic molecules (He et al., 2010). Similarly, cordycepin-mediated apoptosis has been observed in the breast cancer cell line MDA-MB-231 by increasing the mitochondrial translocation of Bax and the release of cytochrome C, followed by the activation of caspases-9 and -3 (Choi et al., 2011). The apoptotic effect of cordycepin has also been investigated in human

![Fig. 1. Illustration of the various interactions of cordycepin in biochemical processes, including nucleic acid synthesis, platelet aggregation, metastasis, inflammatory reactions, apoptosis and cell cycle signaling.](image)
leukemia cells (U937 and THP-1) through the reactive oxygen species (ROS)-mediated activation of caspases (-3, -8 and -9) and the cleavage of the poly (ADP-ribose) polymerase protein (PARP) by cordycepin, leading to apoptotic cell death in human the neuroblastoma SK-NBE(2)-C (CRL-2268) and human melanoma SK-Mel-2 (HTB-68) cell lines. Furthermore, Lee et al. (2012) demonstrated the anti-proliferative effect of cordycepin in human breast cancer cells through the induction of DNA damage, PARP cleavage and the phosphorylation of ATM, ATR and histone γH2AX.

In addition to its involvement in apoptotic pathways, cordycepin is also known to arrest cell cycle at certain check-points. The cell cycle is regulated by cyclin-dependent kinases (CDKs), and cyclins and cyclin-dependent kinase inhibitors (CDKIs) in turn, positively and negatively control the CDKs. Studies of the cell cycle in a human oral squamous cancer cell line (OEC-MI) demonstrated that cordycepin decreased the percentage of G1 phase cells in a dose dependent manner, whereas the percentage of G2M and sub-G1 phase cells was increased (Wu et al., 2007). Another study reported the anti-cancer activity of cordycepin in human bladder carcinoma cell lines (5637 and T-24) through the activation of JNK and cell cycle arrest at G2/M transition via the inhibition of the cyclin B/CDC-complex and the up-regulation of P21WAF1 expression (Lee et al., 2009). Similar results were observed in a study in which colon cancer HCT116 cells were treated with cordycepin (E.J. Lee et al., 2010; S.J. Lee et al., 2011). In 11z human immortalized epithelial endometriotic cells (Imesch et al., 2011), cordycepin demonstrated an anti-proliferative effect through the up-regulation of p27KIP1 via the Ras/ERK1 signaling pathway. Therefore, the induction of apoptosis (Fig. 3) and cell cycle arrest (Fig. 4) comprises of an important mechanism in many anti-cancer drugs, including cordycepin.

The first in vivo study to demonstrate the anticancer properties of cordycepin was performed in mice and demonstrated that cordycepin leads to increased survival time in mice bearing the Ehrlich mouse ascites tumor (Jagger et al., 1961)). Furthermore, a phase 1 clinical trial was completed (Dec 1997–Mar 2001) using cordycepin and pentostatin to treat patients with refractory acute lymphocytic or chronic myelogenous leukemia (NCT00003005). Phase 2 clinical trials are ongoing, and the last update was in Jan 2009 (NCT00709215). Despite these promising results, a number of limitations are associated with the application of cordycepin in clinical settings because it requires the co-administration of ADA inhibitors, such as cladribine/deoxycoformycin (dcm)/coformycin (cf). However, research is ongoing to identify a less toxic, synergistic co-drug for cordycepin that works via mechanisms other than adenosine deaminase resistance (Janek et al., 2007; Cui et al., 2013).

Anti metastatic activity

The major cause of cancer mortality is the metastasis of the cancer from the native site to other parts of the body. Metastasis typically includes the detachment of tumor cells from the primary site, invasion through the extracellular matrix (ECM), followed by invasavation, survival and extravasation from the blood vessels and finally, invasion of the ECM of distant target organs. The ECM is made up of type IV collagen, laminin, heparan sulfate, proteoglycan, nidogen and fibronectin (Nakajima et al., 1987). Metalloproteinases (MMPs) have been shown to play a critical role in the degradation of the ECM. MMPs are a family of zinc- and calcium-dependent endopeptidases that can be divided into four subclasses based on their ECM specificity, including collagenases, gelatinases, stromelysins and matrikylins. Among the MMP family, MMP-2 and 9 are the two key enzymes known to degrade type IV collagen, which is an essential component of the basement membrane. The elevated expression of these MMPs has been functionally correlated with elevated tumor invasion capacity in various cancers, including breast cancer (Egeblad and Werb, 2002; Hunter et al., 2008). The activity of MMPs can be regulated by...
several mechanisms (Woessner, 1991), such as transcription inhibition, pro-enzyme activation or MMP inhibitors, i.e., tissue inhibitors of metalloproteinases (TIMPs). Cytokines and TPA have been known to induce MMP-9 synthesis and secretion via the activation of transcription factors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) during tumor invasion (Chung et al., 2004; Hong et al., 2005; Woo et al., 2005). Cordycepin has been known to inhibit MMP expressions levels (Fig. 5) through the deactivation of AP-1 via the MAPK signaling pathway (Noh et al., 2010). A study demonstrated that cordycepin blocks TNF-α-induced MMP-9 expression by decreasing the DNA-binding activity of transcription factors, such as NF-κB and AP-1 (E.J. Lee et al., 2010; S.J. Lee et al., 201). Yet another study concluded that the combination of cisplatin and cordycepin might act as a potent inhibitor of peritoneal tumor dissemination and VEGF expression (Cai et al., 2011). Furthermore, it has been suggested that cordycepin inhibits the migration and invasion of LNCaP (human prostate carcinoma) cells by down regulating the activity of TJs (tight junctions) and MMPs, possibly in association with the suppression of Akt activation (Jeong et al., 2012).

Anti-platelets aggregation

Platelets are known to regulate tumor angiogenesis, growth and metastasis. Earlier evidence suggested that cancer cells have the ability to activate platelets, which facilitate their survival in the blood circulation during hematogenous metastasis by preventing tumor cell lysis by natural killer (NK) cells and cytotoxic lymphocytes (Tsuruo and Fujita, 2008; Goubran and Burnouf, 2012). A variety of molecular mechanisms (Fig. 6), such as GPIIb/IIIa, GP Ib-IX-V, P2Y receptors, and PAR receptors, have been proposed to describe tumor cell-induced platelet aggregation (TCIPA) (Jackson, 2007; Bambace and Holmes, 2011). Cho et al. (2006, 2007a) reported that cordycepin inhibits collagen-induced platelet aggregation by lowering [Ca2+]i and thromboxane A2 [TXA2] and through the up-regulation of intracellular levels of cAMP and cGMP. Furthermore, the study demonstrated that 500 μM cordycepin blocked the up-regulation of [Ca2+]i and TXA2 by 74% and 46%, respectively. Another study demonstrated that cordycepin exerted a potent inhibitory effect on thapsigargin and U46619-elevated

Fig. 4. Cordycepin has been shown to arrest cell cycle, which is regulated by cyclin dependent kinases (CDKs). Cyclins and cyclin-dependent kinase inhibitors (CDKIs), in turns, positively and negatively control the CDKs. Cordycepin clearly exhibits an anti-proliferative effect through the up-regulation of p21 and p27 and the down-regulation of cyclin D, E and B, followed by a reduction in retinoblastoma protein (pRb) phosphorylation.

Fig. 5. The inhibitory effect of cordycepin on tumor metastasis through the MAPK signaling pathway. MAPK is known to activate the AP-1 molecule, which in turn positively controls the expression of matrix metalloproteinases.
intracellular Ca$^{2+}$, which is known to induce a wide variety of ligands that further facilitate platelet aggregation (Cho et al., 2007b). In addition to [Ca$^{2+}$]$^{2+}$ and TXA2, cordycepin has been known to reduce the hematogenic metastasis of cancer cells via the inhibition of adenosine diphosphate (ADP), which is a potent inducer of platelet aggregation during TCIPA (Yoshikawa et al., 2009).

**Anti-inflammatory activity**

It is understood that inflammatory activities are related to cancer progression. Cancer cells are known to express variety of cytokines, chemokines and their receptors, which play an important role in mediating inflammatory responses (Arias et al., 2007; Nelson et al., 2004; Farrow et al., 2004). Many anti-cancer compounds can also be used to treat inflammatory diseases (Rayburn et al., 2009). Previous studies have reported that the expression of cytokines, such as IL-6, IL-8, G-CSF, IFN-γ, and MIP-1β, is up-regulated in carcinoma (Chavey et al., 2007; Wang et al., 2009). Therefore, to develop a potent strategy to tackle and prevent cancer, it is essential to inhibit inflammatory mediators. Kim et al. (2006) evaluated the anti-inflammatory effect of cordycepin in LPS-stimulated macrophages by inhibiting nitric oxide (NO) production. The study suggested that cordycepin downregulates iNOS, COX-2 and TNF-α gene expression via the suppression of NF-κB activation and Akt and p38 phosphorylation. Similar studies have shown that cordycepin significantly attenuated the release of inflammatory mediators, such as NO, PGE2, TNF-α, and IL-1β in LPS-induced microglia cells by inactivating NF-κB through the inhibition of kB-α degradation (Jeong et al., 2010). In lipo-polysaccharide-stimulated macrophages (Shin et al., 2009), cordycepin suppressed the gene expression of M1 cytokines (IL-1β) and TNF-α and chemokines (CX3CR1 and RANTES), whereas the expression of M2 cytokines (IL-10, IL-1ra, TGF-β) was increased. Furthermore, cordycepin has been shown to increase the expression of the interleukin-10 protein in human peripheral blood mononuclear cells, which is known to inhibit the secretion of proinflammatory and inflammatory cytokines (Moore et al., 1993; Armstrong et al., 1996; Cunha et al., 1992; Mertz et al., 1994; Dokka et al., 2001; Zhou et al., 2002). Rao et al. (2010) demonstrated that cordycepin is a potent inhibitor of free radical NO and cytokine (TNF-α and IL-12) production. The study suggested that cordycepin might be useful for the prevention of cancer by acting as an anti-inflammatory agent. Kim and colleagues demonstrated in a mouse model that cordycepin blocks acute lung inflammatory (ALI) responses and the expression of related genes, including adhesion molecules (ICAM and VCAM), cytokines/chemokines and the chemokine receptor CXCR2. The study suggested that cordycepin blocks LPS-induced VCAM expression in A549 cells through a remarkable reduction in p65-NF-kB, which is directly linked to PARP inhibition (Kim et al., 2011).

**Derivatives of cordycepin**

The intrinsic resistance of adenosine deaminase (ADA) to cordycepin has become a serious issue for the scientific community. ADA is an important enzyme involved in purine metabolism and deaminates adenosine to the inactive metabolite inosine (Saboury et al., 2002). Because of the similarity between cordycepin and adenosine, cordycepin is rapidly metabolized by ADA; therefore, a high therapeutic dose might be required, which could be therapeutically unacceptable. An approach to tackle this problem is to generate structurally different derivatives of cordycepin, which not only enhance the potency but also provide resistance towards ADA. Researchers have reported the production of a cordycepin 2-5A (2-5 linked oligoadenylate) analogue, namely ppp5’(3’dA)2‘pp5’(3’dA)2‘pp5’(3’dA), in lysed rabbit reticulocytes and determined the analogue to be a more potent inhibitor of protein synthesis than the 2-5A trimer triphosphate. It has also been reported that the “core” of the cordycepin analogue, i.e., (3’dA)2′−5’(3’dA)2′−5’(3’dA), similar to the core of 2-5A itself, could prevent the transformation of human lymphocytes infected with Epstein–Barr virus (Doetsch et al., 1981a,b) and could be used to supplement the treatment of cells with interferon. Sawai et al. (1983) prepared dicyclohexylcarbodiimide-induced cordycepin 5′-monophosphate and demonstrated the importance of the 5′-hydroxyl groups in 2-5A for the activation of the 2-5A-dependent endonuclease. The cordycepin analogue and its 5′-monophosphate derivative were shown to exhibit pronounced anti-human immunodeficiency virus type 1 activity in vitro (Muller et al., 1991). Wei et al. (2009) synthesized four
N-acyl-cordycepin derivatives containing a normal alky chain, which not only protected its primary amine from rapid oxidation but also improved its bioavailability and pharmacological activity.

Conclusions and future perspective

Cordyceps are an excellent source of bioactive metabolites that exhibit many clinically approved benefits for human health. Because people have an inclination towards natural/herbal therapies, the use of Cordyceps as a natural medicinal mushroom is inevitable. The challenge is to understand in depth the key factors influencing the growth and cultivation of this fungus. Rigorous efforts will be required to produce this mushroom in enough bulk to obtain sufficient amounts of bioactive compounds, including cordycepin, from its fruiting bodies or mycelial extracts. Modern biotechnological tools will be needed to enhance further the bioactive potential of the metabolite.

Looking at the structure of cordycepin, one can imagine that it has a strong tendency to form transition metal complexes in the form of di, tri and tetra dentate ligands; metals can accommodate a donor atom’s lone pair of electrons into their empty d orbital and cordycepin has five N and three O atoms (Tuli et al., 2013). These complexes can be analyzed further using modern spectroscopic tools, which in turn may improve the bioactivity of the compound. Future studies should focus on the synergistic association of cordycepin with metal-containing anti-cancer drugs (Cai et al., 2011). Additionally, drug delivery strategies using nano-particles containing cordycepin are promising tools for assessing the therapeutic potential of this bio metabolite.

To counter anti-cordycepin resistance, there is a strong need to generate structurally different derivatives of cordycepin that will not only enhance the potency but also provide resistance towards ADA. More sincere efforts will be required to identify most of the pharmacologically active compounds in Cordyceps and to understand their structure-function relationship as well to realize the full potential of this wonderful mushroom for commercialization and ethnopharmacological use.

Conflict of interest statement

There are no potential conflicts of interest among the authors regarding the publication of this manuscript.

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