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Review Article

Functional study of *Cordyceps sinensis* and cordycepin in male reproduction: A review

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ABSTRACT

Cordyceps sinensis has various biological and pharmacological functions, and it has been claimed as a tonic supplement for sexual and reproductive dysfunctions for a long time in oriental society. In this article, the *in vitro* and *in vivo* effects of *C. sinensis* and cordycepin on mouse Leydig cell steroidogenesis are briefly described, the stimulatory mechanisms are summarized, and the recent findings related to the alternative substances regulating male reproductive functions are also discussed.

1. Introduction

Cordyceps sinensis (CS) is a fungal parasite on the larvae of Lepidoptera. In late autumn, the fungus attacks the caterpillar and leisurely devours its host. By early summer of the following year, the fungal infestation has killed the caterpillar and the fruiting body protrudes from its head. Because of its particular life cycle, it is called the *winter-worm, summer-plant* in Chinese [1–3]. CS has long been used as a herbal tonic in

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traditional Chinese medicine to treat many illnesses in the oriental society. Nevertheless, the supply of CS is inadequate for the demand because of its low yield in a high-altitude area where it cannot be easily harvested. However, the mycelium of the fungus has been cultured and the dried powder of the mycelium is commercially available [1–3].

CS contains complex materials, including cordycepin [4–6], modified nucleosides [7,8], polysaccharides [9,10], and sterols [11,12]. Cordycepin, or 3′-deoxyadenosine, is a major bioactive component found in CS. Due to the absence of oxygen in the 3′ position of its ribose moiety and its similarity to adenosine (Figure 1) [6], some enzymes cannot discriminate between the two. Therefore, it can participate in certain biochemical reactions. For example, cordycepin could incorporate into an RNA molecule causing the premature termination of its synthesis, and has a wide range of biological effects in the regulation of inflammation and platelet aggregation [6,13,14]. Studies have demonstrated that CS has multiple pharmacological activities, including modulation of immune responses [15,16], inhibition of tumor growth [11,17,18], decrease of blood pressure [19,20], increase of hepatic energy metabolism and blood flow [21], improvement of bioenergy in liver [22], induction of cell apoptosis [23], and secretion of adrenal hormones [24].

2. Steroidogenic pathway related to male reproduction

In the male reproductive system, gonadotropin-releasing hormone from hypothalamus stimulates anterior lobe of pituitary gland to release luteinizing hormone (LH) [25]. It is well established that steroidogenesis in Leydig cells is regulated by LH/chorionic gonadotropin (CG). LH binds to its receptors in Leydig cells to activate G-proteins and, in turn, adenylate cyclase (AC), which can increase intracellular cyclic adenosine monophosphate (cAMP) formation [26]. The cAMP will then stimulate protein kinase A (PKA), which will phosphorylate some proteins and/or induce *de novo* synthesis of proteins, such as steroidogenic acute regulatory (StAR) protein [27]. StAR protein is considered as a rate-limiting step of steroid biosynthesis since it can facilitate the transfer of free cholesterol from cytoplasm into the inner membrane of mitochondria, where cytochrome P450 side-chain cleavage enzyme converts cholesterol to pregnenolone [28]. Pregnenolone will then be transported to the smooth endoplasmic reticulum, where contains 3β-hydroxysteroid dehydrogenase (HSD), 17α-hydroxylase, 20 α-hydroxylase, and 17β-HSD steroidogenic enzymes, to be processed to become testosterone, an essential steroid hormone for reproduction in men [25].

Although PKA-mediated protein phosphorylation is undoubtedly important in regulating steroid synthesis, other signaling systems have also been implicated. It has been shown that the activation of protein kinase C (PKC) signal pathway can strongly modulate Leydig cell steroidogenesis [29]. Likewise, evidence indicates that calcium is also involved in steroidogenesis. It has been shown that the removal of extracellular calcium, or the addition of calmodulin antagonist or calcium channel blocker blunts Leydig cell steroidogenesis [30,31].

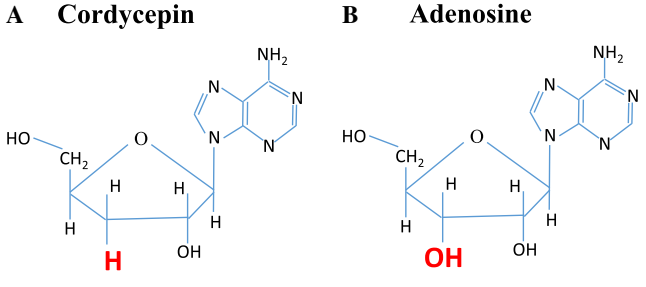

Figure 1 – The difference in the chemical structures of bioactive compounds, (A) cordycepin and (B) adenosine [6].

3. CS stimulates mouse Leydig cell steroidogenesis

3.1. *CS stimulates testosterone production in purified mouse Leydig cells*

It is important to determine whether CS can stimulate testosterone production by mouse Leydig cells. Thus, the mouse Leydig cells are purified through Percoll gradient with purity reaching around 90%. These purified normal mouse Leydig cells are then treated with CS at different dosages for different time durations, and testosterone levels in media are evaluated. The results demonstrate that testosterone production is significantly elevated by the treatment of increasing dosages of CS (0.1–10 mg/mL), and reaches a maximum at 3 mg/mL CS in a 3-hour treatment as compared to control [32].

3.2. *CS fractions stimulate testosterone production in purified mouse Leydig cells*

In previous results, CS stimulated *in vitro* steroidogenesis in purified normal mouse Leydig cells [32]. What remains elusive, however, is which component of CS is responsible. Thus, a search for the components in CS with *in vitro* stimulatory effects on mouse Leydig cells is required. The purified mouse Leydig cells are treated with CS and its water-extracted fractions; F1 (water-soluble polysaccharide), F2 (water-soluble protein), and F3 (poorly water-soluble polysaccharide and protein); to determine the *in vitro* effects of CS fractions on steroidogenesis. In preparation of CS fractions, 100 g crude CS was extracted with 800 mL distilled water and shaken at 37°C for 72 hours. The solution was then centrifuged at 13,000× *g* at 4°C for 30 minutes to collect the pellet (F3). The supernatant was applied into G150 gel filtration column (3 cm × 100 cm) with 50mM acetonitrile buffer at pH 6.0. Two peaks were collected and the first peak was assigned as F1 and the second peak was assigned F2. The yield percentages of F1, F2, and F3 were 1.69%, 13.46%, and 84.85%, respectively. The results illustrate that F2 and F3 significantly stimulate *in vitro* testosterone production in purified mouse Leydig cells in dose- and time-dependent relationships with maximal responses at 3 mg/mL for 3 hours [33].