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Antioxidant and Immunopotentiating Effects of *Cordyceps* Mycelium Extract, Chicken Essence, and Their Combination in Experimental Models

Hoi Yan Leung[1], Choly Tat Ming Yan[2], Kam Ming Ko[1*]

[1]Division of Life Science, Hong Kong University of Science & Technology, Hong Kong SAR, China
[2]Royal Medic Group Holding Limited, Hong Kong SAR, China
Email: *bcrko@ust.hk

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Abstract

Cordyceps mycelium extract (Cs-4), Chicken Essence (CE), and their combination were investigated for antioxidant and immunomodulatory activities in mouse models. Long-term treatment with Cs-4 or CE at equivalent human doses improved antioxidant status in various tissues, as evidenced by the enhancement of mitochondrial glutathione redox status in the brain, liver, heart, and kidney of mice. Cs-4 or CE treatment also produced an immunomodulatory action, as indicated by the potentiation of Concanavalin A-/lipopolysaccharide-induced proliferation of mouse splenocytes *ex vivo*. While doubling of the equivalent human doses of Cs-4 and CE did not further enhance their antioxidant or immunopotentiating effects, the combined treatment with Cs-4 and CE at their respective equivalent human doses produced an additive effect, with the extents of stimulation being larger than those produced by Cs-4 or CE alone. The results have demonstrated for the first time that the combined use of Cs-4 and CE can produce an additive effect on both antioxidation and immunopotentiation that cannot otherwise be achieved by increasing the equivalent human doses of Cs-4 or CE alone.

Keywords

Cordyceps, Chicken Essence, Glutathione Redox Status, Immunopotentiation, Additive Effect

1. Introduction

Cordyceps sinensis (Berk.) Sacc (*C. sinensis*) is a fungal parasite on a larva of *Lepidoptera*. It is known as Dongchongxiacao (winter-worm summer-grass) in Chinese. Cs-4 is a fermentation product derived from *Cordyceps* mycelium cul-

tures. According to the State Pharmacopoeia Commission of People Republic of China 2005, *C. sinensis* can be used for the invigoration of body functions, health preservation, and reduction of fatigue. Pharmacological studies have also indicated that *Cordyceps* can produce antioxidant, antitumor and immunomodulating effects in various experimental models [1] [2].

Chicken Essence (CE) is regarded as a nutritious food supplement in ethnic Chinese societies. It is made by heating a whole chicken at a high-temperature, followed by centrifugation, vacuum concentration, and sterilization of the extract. Research studies have demonstrated the beneficial effects of CE in enhancing immunity, producing anti-fatigue and anti-stress effects, and stimulating metabolism, etc. [3]-[8]. CE contains several distinctive nutrients such as imidazole dipeptide (carnosine and anserine), taurine, polypeptides, minerals, trace elements, and several amino acids. Imidazole dipeptides are well known natural antioxidants that have been shown to suppress oxidative stress in various organs in experimental animals [3] [9] [10] [11].

With the global trend of preventive health, antioxidant enhancement and immunomodulation represent a major strategy in health-promotion that leads to improved quality of life and disease prevention. People commonly take more than one type of health product for safeguarding health. In the present study, we aimed to investigate: 1) the antioxidant effects of Cs-4, CE, and their combination on various tissues and 2) the immunopotentiating effect of Cs-4, CE, and their combination in mice.

2. Materials and Methods

2.1. Animal Care and Samples

Male adult Institute of Cancer Research (ICR) mice were obtained from the Laboratory Animals Facility at the Hong Kong University of Science & Technology (HKUST). They were maintained under a 12-h dark/light cycle at about 22˚C and allowed food and water *ad libitum*. All animal experimental protocols were approved by the Animal Ethics Committee of HKUST (Protocol number: RD2001).

Cs-4 extract and CE, which are commercial health products, were manufactured and supplied by Royal Medic Group Limited, Hong Kong.

2.2. Antioxidant Effect

Animal model

Male adult ICR mice (~8 weeks of age; 20 - 25 g) were randomly assigned to 9 groups, of 6 animals each. The tested sample/vehicle was administered by gavage at 3 increasing doses (Cs-4, 0.125, 0.25, 0.5 g/kg; CE, 6.15, 12.3, 24.6 mL/kg; Cs-4/CE, 0.124 g/kg/0.65 mL/kg, 0.25 g/kg/12.3 mL/kg) for 14 consecutive days.

Mitochondrial glutathione redox status

Twenty-four hours after the last dosing, various organs including the brain, heart, liver, and kidney were isolated from ketamine/xylazine-anesthetized mice,

6and the mitochondrial fraction was isolated by differential centrifugation [12]. Mitochondrial glutathione redox status was assessed by estimating the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio [13].

2.3. Immunomodulating Effect

Concanavalin A (Con A)/Lipopolysaccharide (LPS)-induced blastogenesis of mouse splenocytes *ex vivo*

Male ICR mice (25 - 30 g) were randomly divided into 9 groups of 6 animals. The tested sample/vehicle was administered by gavage at 3 increasing doses for 14 consecutive days, as described above. Twenty-four hours after the last dosing with the test sample, animals were killed by cervical dislocation, and splenic tissues were taken under aseptic conditions for isolation of splenocytes, as described below. Murine splenocytes (10^6 cells) were cultured in a medium with or without Con A/LPS in a final volume of 100 μL. Con A/LPS was added at final concentrations of 0.5, 1, 2, 4, and 7.5 μg/mL.

Isolation of murine splenocytes

All procedures were conducted under aseptic conditions. Splenic tissues were obtained from male ICR mice (25 - 30 g), and isolated splenocytes were resuspended in RPMI-1640 medium supplemented with 10% HIFBS at a concentration of 1×10^7 viable cells/mL. The viability of isolated splenocytes was determined by Trypan blue exclusion.

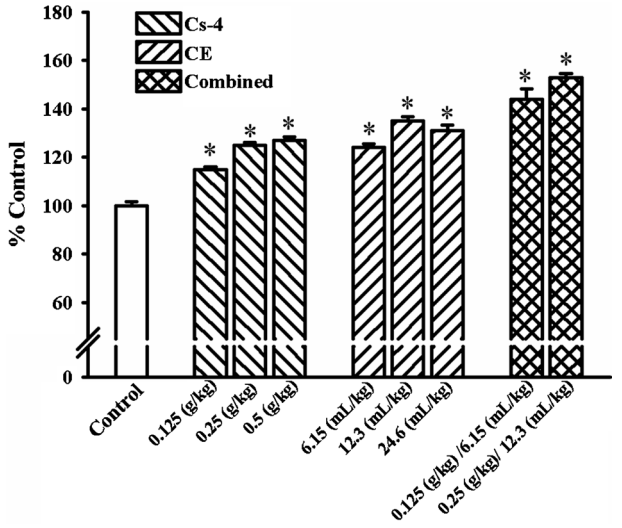
Measurement of splenocyte proliferation

Splenocytes were cultured for 72 h at 37˚C in a humidified atmosphere of 5% CO_2 in atmospheric air. At the end of the culture period, the extent of splenocyte proliferation was determined by a colorimetric assay using a tetrazolium salt (MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide)-based cell proliferation kit. The extent of Con A/ LPS-stimulated proliferation of isolated splenocytes was estimated by computing the area under the curve (AUC) of a graph plotting the percentage of initial absorbance (mean absorbance of cells stimulated with Con A or LPS/mean absorbance of cells not stimulated with Con A or LPS × 100%) against Con A/LPS concentrations. The extent of Con A/LPS-stimulated proliferation of isolated splenocytes was estimated by comparison with the control [14].

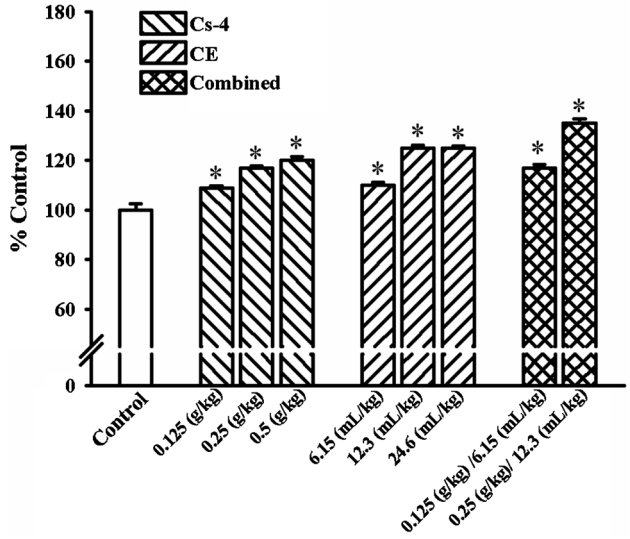
3. Results

3.1. Enhanced Mitochondrial Glutathione Redox Status in Various Tissues

Long-term treatment with Cs-4 (0.25 g/kg ×14 days) or CE (12.3 mL/kg × 14 days) (*i.e.*, at respective human equivalent doses) increased the mitochondrial GSH/GSSG ratio (brain, 25% or 35%; heart, 17% or 25%; kidney 17% or 14%; liver 19% or 17%) (**Figure 1**). Doubling of equivalent human doses for Cs-4 or CE did not cause further increases in mitochondrial GSH/GSSG ratio in any of the tested tissues. The combined treatment with Cs-4 and CE at human equivalent


Cs-4
CE
Combined
% Control
180
160
140
120
100
80
60
0
Control
0.125 (g/kg)
0.25 (g/kg)
0.5 (g/kg)
6.15 (mL/kg)
12.3 (mL/kg)
24.6 (mL/kg)
0.125 (g/kg) /6.15 (mL/kg)
0.25 (g/kg)/ 12.3 (mL/kg)

(a)


Cs-4
CE
Combined
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180
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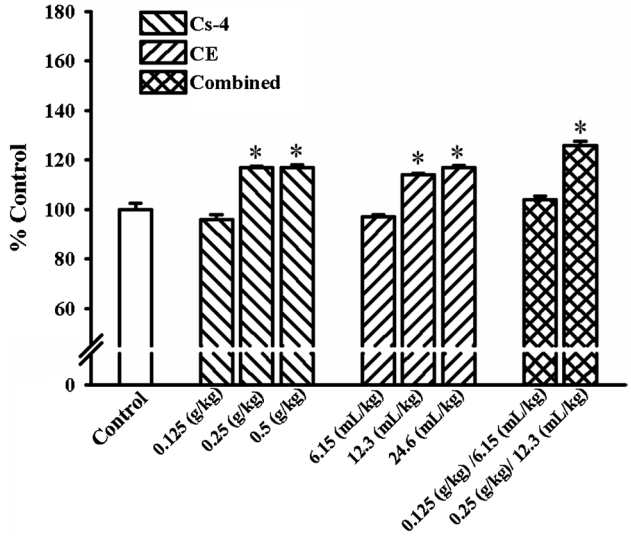
(b)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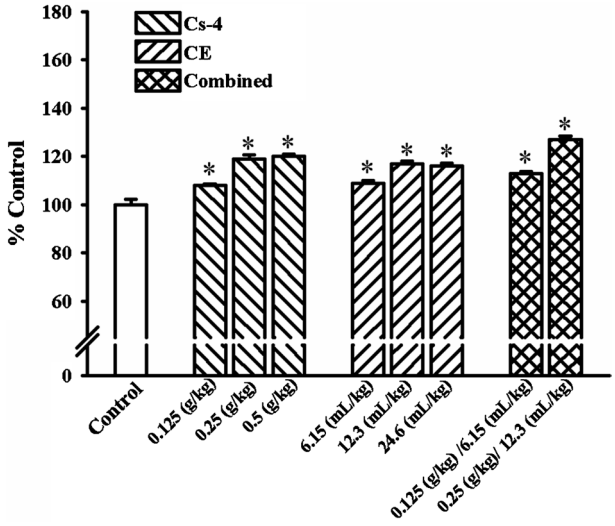


(d)

Figure 1. The effect of Cs-4, CE and their combination on mitochondrial glutathione redox status of various organs in mice. Control values of mitochondrial GSH/GSSG ratio (average ± S.E.M.) of each organ were: brain, 31.3 ± 0.69; heart, 10.5 ± 0.22; kidney, 11.0 ± 0.21; liver, 23.2 ± 0.47. *Significantly different from control (p-value < 0.05). (a) Brain; (b) Heart; (c) Kidney; (d) Liver.

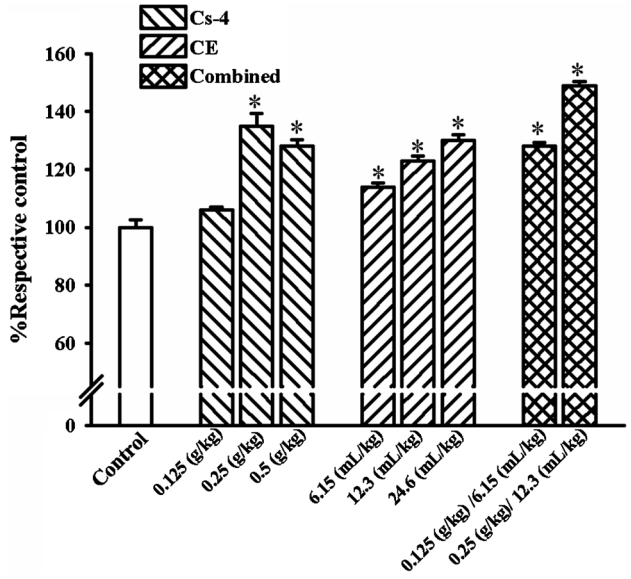
doses further enhanced the mitochondrial glutathione redox status in various tissues of mice, as evidenced by increases GSH/GSSG ratio in an additive manner, with values well above those produced by doubling of the human equivalent dose of Cs-4 or CE alone (brain, 53% vs 27% or 31%; heart, 35% vs 20% or 25%; kidney, 26% vs 17% or 17%; liver, 27% vs 20% or 16%) (**Figure 1**).

3.2. Enhanced Adaptive Immunity

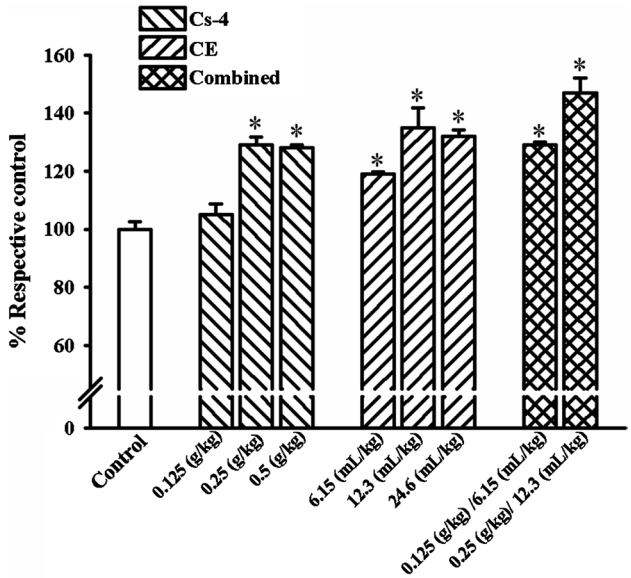
Both long-term Cs-4 or CE treatments at equivalent human doses potentiated the adaptive immune response, as evidenced by increases in the extent of Con A-stimulated (T cells) (35% and 23%, respectively) and LPS-stimulated (B cells) (29% and 35%) proliferation of mouse splenocytes *ex vivo* (**Figure 2**). Doubling of the euivalent human doses for Cs-4 or CE did not cause any further enhancement in Con A- or LPS-stimulated splenocyte proliferation in mice. Combined treatment with Cs-4 and CE at human equivalent doses further potentiated the Con A-/LPS-induced splenocyte proliferation in an additive manner, with the extents of stimulation being well above those produced by doubling the human equivalent dose of Cs-4 or CE alone (49% vs 28% or 30% and 47% vs 29% or 32%, for T cells and B cells, respectively) (**Figure 2**).

4. Discussion

Cs-4, an extract of *Cordyceps* mycelium culture, has long been used for its immunomodulatory action. It has recently been demonstrated that Cs-4 treatment can produce anti-inflammatory effects in allergic rhinitis and asthma in rodent models [15]. CE, which is a health product based on traditionally made chicken



(a)



(b)

Figure 2. The effect of Cs-4, CE and their combination on Con A/LPS-induced blastogenesis of mouse splenocytes *ex vivo*. Control value (AUC) (average ± S.E.M.) were: Con A-induced, 1465 ± 30.00, LPS-induced, 1567 ± 41.75. *Significantly different from control (p-value < 0.05). (a) Con A; (b) LPS.

broth is commonly used for counteracting stress and fatigue, as well as for cognitive enhancement in individuals under stressful situations [6]. Contemporary strategies for safeguarding health emphasize the enhancement of antioxidant and immune functions in the body. In the present study, we endeavored to investigate the effect of long-term treatment with Cs-4 and CE on mitochondrial glutathione redox status, which is a crucial determinant of cellular protection against oxidative stress [16], in various mouse tissues, and Con A-/LPS-induced proliferation of mouse splenocytes (for T cells and B cells, respectively), which

provides a measure of adaptive cell-mediated immunity [17] [18].

Both long-term Cs-4 and CE treatments at equivalent human doses enhanced mitochondrial glutathione redox status in the brain, heart, kidney, and liver tissues of mice. The beneficial effect of the enhancement in mitochondrial glutathione redox status was reflected in the protection afforded by Cs-4 and CE, as well as their combination, against carbon tetrachloride-induced liver oxidative injury in mice, wherein the extent of hepatoprotection correlated well with the degree of enhancement of hepatic mitochondrial glutathione redox status (data not shown). Interestingly, while doubling of the equivalent human dose of Cs-4 or CE treatment did not result in any further enhancement of mitochondrial glutathione redox status in mouse tissues when compared with those produced by the equivalent human dose, the combined treatment with Cs-4 and CE at equivalent human doses enhanced the mitochondrial glutathione redox status in an additive manner, with the degree of stimulation being greater than that produced by Cs-4 or CE alone (at doubled equivalent human doses). Both *Cordyceps* extract and CE have been shown to possess free radical scavenging activity in *in vitro* assays [19] [20]. The findings obtained from the present study have for the first time demonstrated the enhancement of mitochondrial glutathione redox status of various tissues by Cs-4 treatment in mice. An adaptive immune response, as assessed by Con A/LPS-induced proliferation of mouse splenocytes *ex vivo*, was found to be potentiated by long-term treatment with Cs-4 or CE. Similarly, the effect of combined Cs-4 and CE treatment at equivalent human doses was greater than that produced by Cs-4 or CE alone, even at doubled equivalent human doses. *Cordyceps* extract and CE have long been known to produce immunopotentiating effects in both *in vitro* and *in vivo* studies [1] [2] [3]. The immunopotentiating effects of Cs-4 and CE are crucial for the restoration of an impaired immune function to a normal level as well as the reduction of recurring infections. Taken together, the finding of the enhancement in antioxidant and immune status by the combined treatment with Cs-4 and CE that can exceed the maximum stimulation achievable by Cs-4 or CE alone suggests a practical approach to optimizing the use of these two health products in safeguarding health.

In conclusion, our results show that long-term treatment with Cs-4 or CE at equivalent human doses produces both antioxidant and immunopotentiaitng effects in mice. The combined treatment with Cs-4 and CE further enhances the beneficial effects, which otherwise cannot be achieved by simply doubling the equivalent human dose of Cs-4 or CE. The present findings have demonstrated for the first time an optimal use of health products for antioxidant function and immunopotentiation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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