Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Ethnopharmacology 100 (2005) 176–179
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Inhibitory effect on proliferation of vascular smooth muscle cells and protective effect on CCl_4-induced hepatic damage of HEAI extract

Won-Sik Choi [a], Chang-Jin Kim [b], Byeoung-Soo Park [c], Sung-Eun Lee [c], Gary R. Takeoka [d], Dong-Goo Kim [e], Xiang LanPiao [f], Jeong-Han Kim [c,*]

[a] *Department of Life Sciences, Soonchunhyang University, Asan, Chungnam 336-745, Korea*
[b] *Department of Medicine, Soonchunhyang University, Asan, Chungnam 336-745, Korea*
[c] *School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea*
[d] *Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, CA, USA*
[e] *H&M Bio Co. Ltd., Chungju Techno-Park, Chungju National University, Chungbuk 380-702, Korea*
[f] *College of Pharmacy, Seoul National University, 151-742, Korea*

Received 7 September 2004; received in revised form 8 February 2005; accepted 17 February 2005
Available online 6 June 2005

Abstract

The effects of methanol extract from *Hericium erinaceus* cultivated with *Artemisia iwayomogi* (HEAI) on proliferation of vascular smooth muscle cells and CCl_4-induced hepatic damage were evaluated. HEAI was shown to have a potent inhibitory effect on the proliferation of vascular smooth muscle cells (VSMCs). Interestingly, a methanol extract of *Hericium erinaceus* showed no inhibitory effect on the proliferation of VSMCs, while a methanol extract of *Artemisia iwayomogi* possessed strong inhibitory effects on the proliferation of VSMCs. Therefore, the inhibitory effects of HEAI may be caused by the changes of chemical components in the culture broth after the addition of *Artemisia iwayomogi* in the HEAI growth media. HEAI also had a strong protective effect on CCl_4-induced hepatic damage in rats. The activity was evaluated using biochemical parameters such as glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (ALP). HEAI treatment caused a significant reduction in the activity of GOT but not of GPT and ALP in comparison with CCl_4 treatment alone. Histopathological studies showed that liver samples treated with HEAI were significantly different when compared to non-treated animals after CCl_4 exposure.

Keywords: Vascular smooth muscle cell; CCl_4; HEAI; *Hericium erinaceus*; *Artemisia iwayomogi*

1. Introduction

Numerous nutritional studies have shown that components present in dietary mushrooms protect against cancer (deVere White et al., 2002; Wasser, 2002) and cause a dramatic reduction in tumor formation when fed to animals (Ghafar et al., 2002; Oshiman et al., 2002). One of primary mechanisms of anticarcinogenic activity of these mushroom constituents is the inhibition of cytochrome P450-dependent monooxygenase activity in the animal liver to reduce activation of procarcinogen compounds to carcinogens (Tsyrlov et al., 1994). It was recently reported that methanol extracts from *Artemisa iwayomogi*, *Ganoderma lucidum*, *Hericium erinaceus*, *Ganoderma lucidum* cultivated with *Artemisa iwayomogi* and *Hericium erinaceus* cultivated with *Artemisa iwayomogi* (HEAI) had inhibitory effects on the biotransformation of aflatoxin B_1 to aflatoxin B_1-8,9-epoxide (Lee et al., 2003). The latter constituent can form a single initial DNA adduct with the guanyl N_7 atom in the DNA sequence. In

* Corresponding author. Tel.: +82 2 880 4644; fax: +82 2 880 4421.
E-mail address: kjh2404@snu.ac.kr (J.-H. Kim).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.02.037

addition, HEAI possessed immunomodulatory properties on the human complement system that plays a pivotal role in the defense system such as anti-complementary activity and interferon-inducing activity (Lee et al., 2003).

Here we investigated the inhibitory effect of HEAI on the proliferation of human VSMCs and the protective effects of HEAI on CCl_4-induced acute hepatic damage in rats.

2. Materials and methods

2.1. Chemicals

The cell culture materials were obtained from Gibco-BRL (Rockville, MD, USA) and other chemical reagents were from Sigma Chemical Co. (St. Louis, MO, USA). Platelet-derived growth factor (PDGF)-BB was from Upstate Biotechnology (Lake Placid, NY, USA).

2.2. Biological materials

Male albino rats were procured from TG Biotech (Taegu, Korea). They were fed commercial diets (Hindustan Lever, Bangalore, India) and given water ad libitum during the experiments. The room temperature was maintained at 25 °C. Methanol extracts from *Hericium erinaceus* and *Hericium erinaceus* cultivated with *Artemisia iwayomogi* were kindly supplied by H&M Bio Co. Ltd. (Chungju, Korea).

2.3. Cell lines and culture conditions

Human aortic vascular smooth muscle cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in smooth muscle media (SmGM) with 10% fetal bovine serum (FBS) at 37 °C in an incubator with a humidified atmosphere of 95% air and 5% CO_2.

2.4. [^{3}H]-thymidine incorporation assay

The effects of HEAI on [^{3}H]-thymidine incorporation into DNA were assessed. Human VSMCs were plated on 24-well culture plates at a density of 50,000 cells/well and allowed to grow for 3–4 days in vascular smooth muscle cell growth media. Smooth muscle cells were placed in serum-depleted media for 24 h. Cells were then exposed to 50 ng/mL PDGF-BB media for 24 h, which caused a robust increase in [^{3}H]-thymidine incorporation in cells not treated with HEAI. Cells were pulsed with 2 μCi/well [^{3}H]-thymidine (Amersham Pharmacia Biotech, Buckinghamshire, UK). The labeling reaction was terminated 4 h later by aspirating the medium and subjecting the culture to sequential washes on ice with 1 × PBS containing 10% trichloroacetic acid and ethanol/ether (1:1, v/v). Acid-insoluble [^{3}H]-thymidine was extracted into 250 μL of 0.5 M NaOH per well. One hundred μL of this solution was mixed with liquid scintillation fluid and measured in a liquid scintillation counter (Beckman LS-3801, Fullerton, CA).

2.5. Cell proliferation assay

Human vascular smooth muscle cell proliferation was measured by determining cell number. For cell counting, human VSMCs were plated on 12-well culture plates, 1×10^5 cells/mL, and cultured at 371×10^5 cells/mL °C for 24 h. Under these conditions, a cell confluence of ∼70% was reached. 12-well plates were pre-cultured in serum-depleted media in the presence or in the absence of HEAI. After 24 h, the cells were stimulated with 50 ng/mL PDGF-BB during a 24–72 h period. The cells were then trypsinized and counted with a hemocytometer under microscopy.

2.6. CCl_4-induced hepatic damage experiments

Three groups (I–III) each for the experiments of six animals weighing between 130 and 160 g were selected. Group I served as control and received orally 0.2 mL of gum acacia daily for 7 days. Group II was similarly treated as group I. Group III was treated with HEAI at a dose of 250 mg/kg daily for 7 days. On the seventh day, carbon tetrachloride (1.25 mL/kg, p.o.) was administered 30 min after the last dose to all rats except those in group I. After 36 h, all the rats were sacrificed under light ether anesthesia, blood was collected in sterile centrifuge tubes and allowed to clot. Serum, separated by centrifuging at 2500 rpm for 15 min, was used for the estimation of serum glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (ALP).

Moreover, the liver was removed, preserved in neutral buffered formalin and then processed for paraffin embedding, following the standard microtechnique. Five micron sections of liver, stained with alum haemotoxylin and eosin, were observed under microscope for histopathological changes.

2.7. Statistical analysis

The experimental results were expressed as mean ± S.D. A one-way analysis of variance (ANOVA) was used for multiple comparisons followed by application of Duncan's test. The data were considered significant if the probability was less than 0.05.

3. Results and discussion

HEAI, described as a methanol extract of *Hericium erinaceus* cultivated with *Artemisia iwayomogi*, inhibited PDGF-BB-stimulation of human vascular smooth muscle cell proliferation as shown in Fig. 1. For in vitro determination of the effect of HEAI on the proliferation of human VSMCs [^{3}H]-thymidine incorporation into cell DNA without PDGF-BB stimulation was used as one index of