Abstract of Clinical Papers on Antrodia Camphorata

1. Nutrition and Cancer

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Induction of Apoptosis by Antrodia camphorata in Human Premyelocytic Leukemia HL-60 Cells

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Antrodia camphorata (A. camphorata) is well known in Taiwan as a traditional Chinese medicine, and it has been shown to exhibit antioxidant effects. In this study, the ability of A. camphorata to induce apoptosis was studied in cultured human premyelocytic leukemia HL-60 cells. Treatment of the HL-60 cells with a variety of concentrations of the fermented culture broth of A. camphorata (25-150 mg/ml) resulted in dose- and time-dependent sequences of events marked by apoptosis, as shown by loss of cell viability, chromatin condensation, and internucleosomal DNA fragmentation. Furthermore, apoptosis in the HL-60 cells was accompanied by the release of cytochrome c, activation of caspase-3, and specific proteolytic cleavage of poly (ADP-ribose) polymerase (PARP). This increase in A. camphorata-induced apoptosis was also associated with a reduction in the levels of Bcl-2, a potent cell-death inhibitor, and an increase in those of the Bax protein, which heterodimerizes with and thereby inhibits Bcl-2. The data suggest that A. camphorata exerts antiproliferative action and growth inhibition on HL-60 cells through apoptosis induction and that it may have anticancer properties valuable for application in drug products.

2: J Agric Food Chem.

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Antioxidative and hepatoprotective effects of Antrodia camphorata extract.

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Antrodia camphorata (A. camphorata) is well-known in Taiwan as a traditional Chinese medicine. The purpose of this study was to evaluate the ability of A. camphorata extracts to protect against oxidative stress in vitro and against carbon tetrachloride (CCl(4))-induced hepatic injury in vivo. An extract of A. camphorata inhibited nonenzymatic iron-induced lipid peroxidation in rat brain homogenates with an IC(50) value about 3.1 mg/mL. It also scavenged the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). The dose of the A. camphorata extract resulting in a decrease of 0.20 in the absorbance of DPPH was about 31 +/- 0.7 microg/mL. Furthermore, an A. camphorata extract dose-dependently (250-1250 mg/kg) ameliorated the increase in plasma aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) levels caused by chronic repeated CCl(4) intoxication in mice. Moreover, A. camphorata extract significantly improved the CCl(4)-induced increase in hepatic glutathione peroxidase, reductase, and CCl(4)-induced decrease in superoxide dismutase activities. It also restored the decrement in the glutathione content and catalase activity of hepatic tissues in CCl(4)-intoxicated mice. Furthermore, it also dose-dependently inhibited the formation of lipid peroxidative products during CCl(4) treatment. Histopathological changes of hepatic lesions induced by CCl(4) were significantly ameliorated by treatment with an A. camphorata extract in a dose-dependent manner. These results suggest that A. camphorata extract exerts effective protection against chronic chemical-induced hepatic injury in vivo, by mediating antioxidative and free radical scavenging activities.

3. Nutrition and Cancer

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Unique Formosan Mushroom Antrodia camphorata Differentially Inhibits Androgen-Responsive LNCaP and -Independent PC-3 Prostate Cancer Cells

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Antrodia camphorata (AC), a precious and unique folkloric medicinal mushroom enriched in polyphenolics, isoflavonoids, triterpenoids, and polysaccharides, has been diversely used in Formosa (Taiwan) since the 18th century. In this study, prostate cancer (PCa) cell lines PC-3 (androgen independent) and LNCaP (androgen responsive) were treated with AC crude extract (ACCE) at 50—200 μg/mL, respectively, for 48 h. At the minimum effective dose 150 μg/mL, LNCaP showed a G1/S phase arrest with significant apoptosis. Such dose-dependent behavior of LNCaP cells in response to ACCE was confirmed to proceed as Akt → p53 → p21 →CDK4/cyclin D1 → G1/S-phase arrest → apoptosis, which involved inhibiting cyclin D1 activity and preventing pRb phosphorylation. In contrast, being without p53, PC-3 cells showed a G2/M-phase arrest mediated through pathway p21 → cyclin B1/Cdc2 → G2/M-phase arrest, however, with limited degree of apoptosis, implicating that ACCE is able to differentially inhibit the growth of different PCa cells by modulating different cell cycle signaling pathways. We conclude that this unique Formosan mushroom, A. camphorata, due to its nontoxicity, might be used as a good adjuvant anticancer therapy for prostate cancers despite its androgen-responsive behaviors, which has long been a serious drawback often encountered clinically in hormonal refractory cases treated by antihormonal therapies and chemotherapeutics.

4. Acta Pharmacologica Sinica

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Modulation of inflammation-related genes of polysaccharides fractionated from mycelia of medicinal basidiomycete *Antrodia camphorata*[1]

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Aim: To investigate the effect of water soluble-ethanol precipitation fraction (AC-1) and alkaline extraction-isoelectric precipitation fraction (AC-2) from *Antrodia camphorata* (Polyporaceae, Aphyllophorales) on lipopolysaccharide (LPS)-induced gene activation in mouse macrophages.

Methods: The AC-1 and AC-2 fractions were prepared, and their effects on LPS-induced gene expression were monitored by Western blotting and RT-PCR.

Results: Our results indicated that AC-2, but not AC-1 dose-dependently (50–200 mg/L) inhibited LPS-induced nitric oxide production as well as the protein and the mRNA expression of the inducible nitric oxide synthase (iNOS) gene. Neither AC-1 nor AC-2 inhibited LPS-induced cyclooxygenase-2 gene expression. Using the cytokine array assay, it showed that AC-2 also had the ability to inhibit LPS-induced the protein expression of interleukin (IL)-6, IL-10, the monocyte chemoattractant protein (MCP)-5, and regulated upon activation, normal T-cell expressed, and

presumably secreted (RANTES). Like iNOS, AC-2 inhibiting LPS-induced IL-6 and IL-10 secretion resulted from inhibiting their mRNA expression.

Conclusion: It was suggested that alkaline extraction-isoelectric precipitated the polysaccharide fraction of *A camphorata* and had the ability to inhibit LPS-induced iNOS, IL-6, IL-10, MCP-5, and RANTES expression in mouse macrophages.

5. PROTEOMICS

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Proteomic analysis of the effect of *Antrodia camphorata* extract on human lung cancer A549 cell

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Antrodia camphorata (niu-chang-chih) is a fungus native to Taiwan that is believed to be effective in preventing diseases. This study demonstrates that 0.2-2% v/v ethanol extracts of *A. camphorata* cultivated by solid-state fermentation (SACE) can effectively impede the proliferation of human non-small cell lung carcinoma A549 cells but not primary human fetal lung fibroblast MRC-5. The results of apoptotic analyses implicate that SACE might trigger the apoptosis in the A549 cells by inducing endoplasmic reticulum stress. Two-dimensional gel maps of non-treated and treated A549 cells were compared using PDQUEST analytical software to discover five statistically significant twofold or above-twofold differentially-expressed protein spots. The five protein spots that were significantly de-regulated were chosen for subsequent identification by high performance liquid chromatography electro-spray tandem mass spectrometry. The five proteins were later identified as human galectin-1, human eukaryotic translation initiation factor 5A, human Rho GDP dissociation inhibitor α, human calcium-dependent protease small subunit and human annexin V. All five proteins were confirmed to be down-regulated by Western blotting. The analytical results of this study help to provide insight into the effect of SACE on the gene expression of the tumor cells.

6. FEMS Microbiology Letters

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Antrodia camphorata prevents rat pheochromocytoma cells from serum deprivation-induced apoptosis

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Antrodia camphorata (*A. camphorata*) is a rare medicinal fungus with antioxidative, vasorelaxtative, anti-inflammatory and anti-hepatitive effects. However, the neuroprotective effect has not been studied. By using serum deprivation-induced apoptosis in neuronal-like PC12 cells as a cell stress model, we found that *A. camphorata* is effective in preventing serum-deprived apoptosis. Inhibitors of both a serine/threonine kinase and a specific protein kinase A (PKA) inhibited the protective effect of *A. camphorata*, indicating that *A. camphorata* prevents serum-deprived PC12 cell apoptosis through a PKA-dependent mechanism. A transcription inhibitor, actinomycin D, and a protein synthesis inhibitor, cyclohexamide, both attenuated the protective effect of *A. camphorata*, indicating a requirement for gene expression for protection by *A. camphorata*. On the other hand, *A. camphorata* also increased phosphorylated CREB, a transcription factor, which is H-89-inhibitable in this study, suggesting the possibility that *A. camphorata* prevents serum deprivation-induced PC12 cell apoptosis through a PKA/CREB-dependent pathway.

7. FEMS Microbiology Letters
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Anti-inflammatory activity of the extracts from mycelia of *Antrodia camphorata* cultured with water-soluble fractions from five different *Cinnamomum* species

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We have previously reported that polysaccharides extracted from fruiting bodies or cultured mycelia of *Antrodia camphorata* exhibit an anti-hepatitis B virus effect. In this study, we intended to elucidate the anti-inflammatory potency of six mycelial extracts, namely PDB-ext, CK-ext, CM-ext, CO-ext, CC-ext, and CKO-ext, isolated from mycelia of *A. camphorata* cultured with six different media including potato dextrose broth (PDB) and five water-soluble fractions from the wood of different *Cinnamomum* species, i.e. *C. kanehirae* (CK), *C. micranthum* (CM), *C. osmophloeum* (CO), *C. camphora* (CC), and *C. kotoense* (CKO), against reactive oxygen species (ROS) production induced by *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) or phorbol 12-myristate 13-acetate (PMA) in peripheral human neutrophils (PMN) or mononuclear cells (MNC). ROS produced by PMN or MNC act as inflammatory mediators and also signal immune responses. Pretreatment with these mycelial extracts (1–50 μg ml^{-1}) concentration-dependently diminished fMLP- or PMA-induced ROS production in PMN or MNC, as measured by lucigenin-amplified chemiluminescence, with 50% inhibition concentrations (IC_{50}) ranging from 2 to 20 μg ml^{-1}. Among these extracts evaluated, CM-ext, CO-ext, or CKO-ext exhibited higher potency than the others. Using high performance liquid chromatography, we identified two lanostane-type compounds, i.e. dehydrosulfurenic acid and 15α-acetyl-dehydrosulfurenic acid, which could be involved in the anti-inflammatory actions of these extracts. The anti-inflammatory actions of these extracts were not due to cytotoxic effects. In summary, these data suggest that extracts from cultured mycelia of *A. camphorata* display anti-inflammatory effects by inhibiting ROS production in human leukocytes at a pharmacologically applicable concentration. The biological activities of these extracts were further promoted when the culture medium was replaced with water-soluble fractions isolated from the wood of CM, CO or CKO.