Anticancer effects and mechanism of triterpenoids from Antrodia camphorata

Yuanchuan He¹, Zenghui Lu¹, Ping Shi¹, Zongjie Zhao², Shijiang Chen¹*  
¹ Chongqing Academy of Chinese Material Medica, Chongqing, China, 404100  
² HongKong Academy of Chinese Medical Science, HongKong, China, 999077

Abstract: Antrodia camphorata is a rare and precious parasitic fungus. A great deal ergostanne and lanostane triterpenoids were isolated and elucidated. This review discussed relationship of chemical structure and bioactivities, anticancer effect and possible mechanism of triterpenoids compounds, and related pharmacological effects, such as anti-inflammatory, immunomodulatory, hepatoprotective and antioxidant activities with various pathways in vitro and animal experiments.

Keywords: Antrodia camphorata; Triterpenoids; Anti-cancer; Anti-inflammatory; Immunomodulatory; Hepatoprotective activity; Antioxidant effect

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* Corresponding Author: Shijiang Chen, shijiangchen@163.com

1. Introduction

Antrodia camphoratais a unique and precious parasitic fungus growing restrictedly on the inner cavity wall of the endemic species Cinnamomum kanehira Hay (Lauraceae) and endangered tree endemic to Taiwan, which has been used as a folk medicine for protection of diverse health-related conditions and treatment of food and drug intoxications, diarrhea, abdominal pain, hypertension, skin itching, and liver cancer [1]. Previous research exhibited triterpenoids rich in ergostane and lanostane isolated from A. camphorata triterpenoids showed anti-cancer, anti-inflammatory, immunomodulatory, hepatoprotective activities and antioxidant effects [2-3].

2. Chemical structure of triterpenoids

Generally triterpenoids is the predominant bioactive constituents in fruiting bodies, which is the main bitter source of A. camphorata, though there is only a little in the solid-state cultivated mycelium and mycelium from submerged cultivations. A large number of triterpenoids isolated from the fruiting body with similar or even the same structures were identified within the last few years [3-4]. A common feature of these triterpenoids is ergostane and lanostane skeleton typical structures (Figure.1). Compared these compounds and theirs bioactivities, there is correlation of substituted groups and pharmacological activities. Triterpenoids showed various cytotoxic potential for different cancer cells line, most of the ergostane-type triterpenoids exhibited anticancer activities, with nontoxic to normal cells. 25R ergostane stereoisomers showed higher activities than the corresponding 25S for antcin B, antcin C, and antcin H. compound 1 and 2 are the derivative of antcin H and antcin B respectively. Compound 3 and 4 are the first pair of cis-trans-isomers of ergostane-type triterpenoids containing an aldehyde group, could be biosynthetic precursors for antcin A. Compound 7-9 and 13-16 are 7 pairs of C-25 epimers. Compound 13 and 16 are 4β and 3β-hydroxylated derivatives of antcin C respectively, compound 10 is 12α-hydroxylated derivative of antcin B [5]. Compounds 6, 10 and 11 have no anti-cancer, anti-inflammatory and anti-insecticidal effects, suggesting R3 and R5 groups affect bioactive effects. Molecular modeling indicates that hydrophilic group C-7 of five antcins contributes to attach glucocorticoid receptorinto nucleus to initiate the suppressing inflammation [6]. Antcin A exhibited significantly higher inhibitory potency than other four antcins, though weaker than ginsenoside Rh2, binding to Na⁺/K⁺-ATPase with the steroidal skeleton structurally upside-down in comparison with ginsenoside Rh2. The inhibitory potency of Antcin A is attributed to steroidal hydrophobic interaction within binding pocket and the formation of three hydrogen bonds between its carbonyl group and two cationic residues around the cavity entrance of Na⁺/K⁺-ATPase. The presence of an additional carbonyl or hydroxyl group at C7 of the other four antcins leads to severe repulsion in the hydrophobic pocket, and thus significantly reduces inhibitory potency [7]. Antcin B, C, H, K and their isomer forms exhibited different anti-proliferative activity against three human leukemia cell lines. The derivatives with carbonyl group at C3 showed cytotoxic with IC₅₀ values ranging from 16.44 to 77.04 μg ml⁻¹ [8]. Compounds 35-38, four newly constituents isolated from A. camphorata, significantly suppressed the NO concentration in LPS-treated RAW 264.7 cell with IC₅₀ values ≤10μM, indicating C-11and C-26 group contribute to activity. However, only derivatives of compound 36 and 38 showed the same anti-inflammatory effect [9].

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Figure 1. Structure of triterpenoids isolated from *A. camphorata*
3. Cytotoxicity on cancer cells

Screening of anticancer compounds has been the focus all over the world, which suppressed cancer cells proliferation and promoted apoptosis. In previous research, Antcin B, Antcin H,Antcin C, Methyl antcinate A(MAA), Methyl antcinate B, Methyl antcinate K, Dehydroeburicoic acid (DeEA,25), Eburicoic acid(28), 15a-acetyl-dehydrodysulfurenic acid (27), Camphoratins B-F; six lanostane triterpenoids and antcamphins A-L exhibited significant selective cytotoxic effects to various cancer cells [4-5, 8, 10-13]. MAA and DeEA could be the most potent anti-cancer agent for different cancer cells. MAA exhibited the most potent spectrum of anti-cancer effects in KB cells, four different oral cancer cell lines (TSCCa, GNM, OC-2, and OEC-M1), Panc-1, BT474, PC-3, OVCAR-3, HeLa, and U2OS cells with high selectivity indices (CC50/IC50) [14]. In oral cancer cells lines OEC-M1 and OC-2, MAA induces tumor growth inhibition via Bax-mediated mitochondrial apoptotic pathway in a dose-dependent manner. MAA increased expression of pro-apoptotic Bax, promoted poly (ADP-ribose) polymerase cleavage, activated caspase-3 and decreased expression of anti-apoptotic Bcl-2 and Bcl-xL [15]. MAA exerts PC-3 cells death through the caspase-dependent cascade and the Box-mediated mitochondrial apoptotic pathway, with the expression of B-cell lymphoma 2 (Bcl-2), Bcl-2 associated X protein (Bear), and poly (ADP-ribose) polymerase (PARP). In human liver cancer cell line HuH7, MAA induced an apoptotic cell death by the appearance ofsub-G1 population, DNA fragmentation, TUNEL positive cells, caspase activation, and triggered the mitochondrial apoptotic pathway as indicted by an increase in the protein expression of Bax, Bak,and PUMA, as well as a decrease in Bcl-XL and Bcl-2 and disruption of mitochondrial membrane potentialand promotion of mitochondrial cytochrome c release, as well as activation of caspas-2, -3, and -9 [16]. Furthermore, MAA exerts cell death through the caspase-dependent cascade and the Bax-mediated mitochondrial apoptotic pathway, not only on liver and oral cancer cells but on other types as well, including prostate cancer, in a dose-dependent manner [14]. MAA showed good inhibitory effects on the self-renewal capability and inhibited cell migration ability in MCF7 sphere cells. MAA also suppressed the expression of heat shock protein 27 and increased the expression of Iκα and p53 [17]. DeEA, which is another most potent anti-cancer agent, enhanced lactate dehydrogenase release in a concentration and time-dependent manner in U87MG cells. DeEA treatment led to a rapid increase of glioblastomas in the necrotic/late apoptotic fraction, but failed to significantly enhance the population of U87MGcells in the hypodiploid fraction. DeEA decreased mitochondrial transmembrane potential and intracellular ATP level, and induced significant cell enlargements, massive cytoplasmic vacuolization and loss of mitochondrial membrane integrity. In addition, DeEA treatment triggered an intracellular Ca2+increase and induced proteolysis of R-spectrin by calpain. DeEA activated DNA damage and apoptosis biomarkers and inhibited topoisomerase II. In HL-60 cells xenograft animal model, DeEA treatment resulted in a marked decrease of tumor weight and size without any significant decrease in mice body weights [13].

Excepted MAA and DeEA, dehydrosulphurenic acid, markedly inhibited growth of human leukemia U937 and pancreatic cancer BxPC3 cells, which resulted in distinct patterns of cell cycle distribution in U937 (G2/M, sub-G1 and polyploidy) and BxPC3 cells (G0/G1 and sub-G1). The modes of cell death in U937 cells include apoptosis and mitotic catastrophe, whereas apoptosis-associated events or necrosis in BxPC3 cells, without mitochondrial membrane permeabilization and caspase dependence. Proteins involving mitotic catastrophe-associated cell death such as cyclin B1 and checkpoint kinase 2 were activated in U937 cells [18]. In HT-29 cells, Antcin B, H and methyl antcinate B were capable of blocking cell cycle progression at the sub-G1 phase and inducing apoptosis through the cleavage of the downstream poly-(ADP-ribose) polymerase, Bcl-2 and procaspase-3. In addition, the combination of these ergostane-triterpenoids showed a potential synergistic cytotoxic effect in HT-29 cells [19].

4. Related anti-cancer effects

Proliferation, infiltration and diffusion of cancer cells are associated with inflammatory, lowered immunity, release of cytokines, liver diseases and another related cancer factors. Most of anticancer components always showed complex bioactivities of related cancer. Excepted cytotoxic effects of cancer cells, most of triterpenoids also exhibited another pharmacological activity as following.

4.1 Anti-inflammatory / Immunomodulatory effects

In peripheral human neutrophils, production of reactive oxygen species including hydrogen peroxide and superoxide anion plays an important role for the killing of microorganisms, but uncontrolled production of these toxic metabolites also damages the surrounding tissues during inflammation. Furthermore, activated neutrophils are more likely to express β2-integrin for their firm adherence to vascular endothelium cells where they subsequently transmigrate to injured tissue releasing hydrolytic enzymes and a large quantity of ROS that is an important inflammatory mediator and also signals immuno-responses. Therefore, anti-oxidant therapy is a comprehensive pharmacological approach in inflammatory disorders. One of the important strategies for the anti-oxidant therapy is to look for natural compounds from plants for the prevention of ROS-induced damage by inflammatory cells. Thirteen triterpenoids were elucidated the
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anti-inflammatory effects by an acute cellular model in isolated peripheral human neutrophils and found that 1-25μM of AntcinB, H, I and K concentration-dependently diminish fMLP- or PMA-induced ROS production with IC50 around 5-20μM. They effectively inhibited the fMLP- or PMA-induced firm adhesion without interfering with the up-expression of surface Mac-1(CD11b/CD18), αβ2 integrin mediating the firm adhesion of neutrophils to endothelium in concentration-dependent manner [20]. Some new lanostanoids significantly suppressed the NO concentration in LPS-treated RAW264.7 cells with IC50 values ≤10 μM [9]. Antcin A could mimics glucocorticoids and triggers translocation of glucocorticoid receptor into nucleus to initiate the suppressing inflammation [6]. Antcin A and B exhibited more inhibition than ibuprofen against fMLP-induced superoxide production with IC50 values less than 10μM [21]. Antcin C, eburicoic acid (EA), DeEA, dehydroeburicoic acid and15α-acetyl-dehydrosulfurenic acid could diminish fMLP- and PMA-induced ROS production in PMN, without cytotoxic effect [22]. EA and DeEA decreased the paw edema at the 4th and 5th h after λ-carrageenan (Carr) administration and increased the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxide (GPx) in the paw edema tissue. EA and DeEA significantly attenuated the malondialdehyde (MDA), nitric oxide (NO), tumor necrosis factor (TNF-α), and interleukin-1β (IL-1β) levels in either edema paw or serum at the 5th h after Carr injection, decreasing carr-induced inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) expression and diminishing neutrophil infiltration into the paw edema[11]. Camphoratins F and J, Antcin A, B, C, methyl Antcinate A and B, methyl 4α-methylergost-8,24(28)-diene-3,11-dion-26-oate exhibited anti-inflammatory NO-production inhibition activities with IC50 values of less than 5μm, and were more potent than the nonspecific NO inhibitor Nω-nitro-L-arginine methyl ester [2, 12]. Four new lanostanoids and lactone derivatives significantly suppressed the NO concentration in LPS-Treated RAW 264.7 cells with IC50 value ≤10μM [9]. Inhibition of ROS production in leukocytes by these active principles could be responsible for the immuno-modulating effects of A. camphorata. For immunomodulatory effects, the results of some lanostane- and ergostane-type compounds tested showed that Antcin C, Sulphurenic, EA and DeEA immunomodulated against reactive oxygen species production induced by fMLP or PMA in peripheral human neutrophils or mononuclear cells [23]. EA and DeEA exhibited anti-inflammatory bioactivities with decreasing carr-induced iNOS and COX-2 expression, significantly attenuating the MDA, NO, TNF-α and IL-1β levels, increase of antioxidant enzyme activity [24]. In addition, methyl antcinate K promoted DC maturation by enhancing the expression of MHC class II, CD86, and reducing the endocytosis. TNF-α, MCP-1 and MIP-1b were secreted by DC after me-AntK treatment, indicating augmentation of innate immunity by Me-AntK. Me-AntK treatment uniquely activated JNK and ERK in DC. Interestingly, the Me-AntK-activated DC induced Ag-specific T-cell proliferation and facilitated Th2 differentiation. This suggests that Me-AntK can potentially be applied to enhance immune responses and modulate DC function in immunotherapy [25].

4.2 Hepatoprotective activity

A. camphorata has been as a hepatoprotective traditional prescription for the treatment of liver disease for long history. Fruiting bodies, mycelium and some substrates isolated from A.camphorata were shown to have protective activity against liver hepatitis, fatty liver and fibrosis induced by acute hepatotoxicity of alcohol, CCl4, and lip polysaccharide. A.camphorata inhibited hepatitis B virus replication and liver cancer cells growth. Some extracts of A.camphorata exhibited angiotensin-converting enzyme (ACE) inhibition activities in spontaneously hypertensive rats and reduce glutathione (GSH)-dependent enzymes such as glutathione peroxidase, glutathione reductase and glutathione-S-transferase. The reported data showed that crude triterpenoids may exert its hepatoprotective effects, through different mechanisms such as scavenging free radicals responsible for antioxidant activity, inhibiting the inflammatory mediators and/or induction of the regeneration of the liver cells. Eburicoic acid and dehydroeburicoic acid, which are lanstone-type triterpenoids isolated from A.camphorata, exhibited hepatoprotective effects against carbon tetrachloride-induced liver damage, preventing the elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and liver peroxides. The activities of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), nitric oxide (NO) production, and tumor necrosis factor-alpha (TNF-α) were decreased after the treatment with these two triterpenoids in CCI4-treated mice. Western blotting revealed that Eburicoic acid and dehydroeburicoic acid significantly decreased inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expressions and increased the expression of cytochrome P450 in CCI4-treated mice [26]. 15-hydroxy-3, 23-dioxolanosta-7,9(11)-dien-26-oic acid decreased the enzymes serum GOT and GPT activities in mice induced by CCI4 [27]. Antrodin B significantly ameliorated cell proliferation, cell migration, suppressed HSC activation marker α-smooth muscle actin expression and ECM components Col1, Col3 and Fn expression, and blocked the phosphorylation of Smad2/3 induced by TGF-β1 in CFSC-8B cells in a dose-dependent manner [28].

4.3 Anti-oxidant activities

Reactive oxygen species (ROS), a highly reactive
molecule, includes hydrogen peroxide (H₂O₂), hypochlorous acid (HClO) and free radicals such as hydroxyl radical (HO•) and the superoxide anion (O₂⁻) [29]. These pathological events play an important role in a wide range of human diseases including cancer, diabetes and cardiovascular diseases [30]. In previous biological studies, the fruiting bodies and mycelia in submerged culture exhibited anti-oxidative effect [21-33]. Antcin A, B, H, I and K concentration dependently diminished fMLP- or PMA-induced ROS production with IC₅₀ around 5-20μM. Antcin A and B exhibited more inhibition than ibuprofen against fMLP-induced superoxide production with IC₅₀ values less than 10μM [21]. AntcinC, eburicic acid (EA), DeEA, dehydrosulfurenic acid and 15α-acetyl-dehydrosulfurenic acid could diminish fMLP- and PMA-induced ROS production in PMN, without cytotoxic effects [20]. Antcin C, sulphurenic, EA and DeEA immunomodulated against reactive oxygen species production induced by fMLP or PMA in peripheral human neutrophils or mononuclear cells [23].

4.4 Others
Methyl antcinate B, Antcin A and K displayed potential anti-Helicobacter pylori pylori activity and its associated inflammation in human gastric epithelial AGS cells by inhibition of adhesion and invasion to AGS cells and NF-κB activation and the subsequent release of interleukin (IL)-8 in a range of concentrations between 0.005 and 0.02 μM. Especially, Methyl antcinate B inhibited the translocation and phosphorylation of CagA and caused a reduction in hummingbird phenotype in HP-infected AGS cells [35-36]. Antcin A is a bi-functional compound that exerts anti-inflammatory effects and that enhances blood circulation via two different molecular mechanisms [7]. Ergostatrien-3β-ol(EK100) isolated from the submerged whole broth of A. camphorata have effect on type 2 diabetes associated with hyperlipidemia in HFD-fed mice by regulation of GLUT4, PEPCK, G6 Pase, SREBP1c, SREBP2, apo A-1, and AMPK phosphorylation [37].

5. Summary and outlook
The features of cancer comprise six biological capabilities acquired during the multistep development of human tumors, including promotion inflammation, infiltrating cells of immune system, associated with fibroblasts and so on. Importantly, inflammation is in some cases evident at the earliest stages of neoplastic progression and is demonstrably capable of fostering the development of incipient neoplasias into full-blown cancers. Additionally, inflammatory cells can release chemicals, notably reactive oxygen species, which are actively mutagenic for nearby cancer cells, accelerating their genetic evolution toward states of heightened malignancy. As such, inflammation can be considered an enabling characteristic for its contributions to the acquisition of core hallmark capabilities [38]. In addition, liver cancer and gastric cancer are often associated with liver disease and Helicobacter pylori infection respectively [39-40]. Recent studies using antioxidants in the appropriate high and repeated doses showed that they actually improve the efficacy of tumor response to chemotherapy and radiological therapy and antioxidants at high doses selectively inhibit the growth of cancer cells without affecting the normal cells [41]. As above, primary pharmacological results of primary screening experiments in vitro exhibited that triterpenoids isolated from A. camphorata showed multiple pharmacological activities, such as cancer prevent, anti-inflammatory activities and hepatoprotective activities, without animal model and preclinical research. According to features of cancer during the multistep development of human tumors, components with complex pharmacological would be focus of anti-cancer compounds and are needed to be research clinical studies to confirm precise mechanism against cancer.

References
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