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Pharmacological Studies of Isoobtusilactone A from *Cinnamomum kotoense with* Anticancer Potential

Chi-Ming Liu[1], Chiu-Li Kao[1], Sheue-Jiun Chen[2], Ya-Fei Kang[2], Wei-Jen Li[2], Hui-Ming Wu[2], Hsin-Liang Chen[2], Hsing-Tan Li[2*], and Chung-Yi Chen[2*]

[1]Department of Nursing, Tzu Hui Institute of Technology, Pingtung County 92641, Taiwan
[2]School of Medical and Health Sciences, Fooyin University, Kaohsiung 83102, Taiwan, R.O.C.

ABSTRACT

Cancer is a complex disease involving unregulated cell growth. Cancer remains the leading cause of death over the world. Isoobtusilactone A, a butanolide, has been first isolated from the ripe berries of Lindera benzoin (Lauraceae). Studies have shown that isoobtusilactone A has potential anticancer activity and induces apoptosis in many cancers. This review describes the pharmacological properties of isoobtusilactone A. This review gathers the information from eletronic and scientific literature database such as Pubmed, Medline, ScienceDirect and so on. Isoobtusilactone A has anticancer activity and modulates multiple targets in cancer cells. The review presents the molecular mechanism of action of isoobtusilactone A in cancer cells and provides information for researchers.

Key Words: Lauraceae; isoobtusilactone A; apoptosis; reative oxygen species

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INTRODUCTION

Isoobtusilactone A (IOA) is a butanolide first isolated from the ripe berries of *Lindera benzoin* (Lauraceae) (figure 1) [1]. IOA is also isolated from plant species of many genera [1-5]. In our lab, we have isolated IOA from the stems of *Cinnamomum reticulatum* Hay [2] and the leaves of *Cinnamomum kotoense* [3]. IOA displays potentially anticancer activity. No attention will be paid on how to isolate the compounds in this review. We will focus on describing pharmacological mechanism of action of isoobtusilactone A in cancer cells.

Cytotoxic activity and Genotoxic activity

IOA exhibits moderate cytotoxic activity in human breast cancer cell (MCF-7, MDA-MB-231) [6], human hepatoma cell (Hep G2) [7-9], human non-small cell lung cancer (A549) [5,10], human larynx carcinoma cell (Hep_2) [11] and murine leukemia cell line (P-388) [5]. The IC_{50} values of IOA are 16.7 μM, 37.5 μM, 2.1 μM, 1.8 μM for HepG2, Hep_2, MCF-7 and MDA-MB-231. Moreover, the IC_{50} values of IOA are 5.29 μM, 5.71 μM, 5.99 μM for 24, 48 and 72 hr, respectively by MTT assay in A549 cells. The genotoxic (DNA damage) activity of IOA was evaluated by comet assay in CHO K1 (Chinese hamster ovary) and HTC (rat hepatoma) cell line [11]. IOA has genotoxic activity by bearing an α-alkylidene-γ-lactone skeleton [11].

Apoptosis

Apoptosis refers to program cell death involving either the mitochondria (intrinsic pathway) or the activation of death receptors (extrinsic pathway). Both pathways induce activation of caspase including initiator caspases (caspase-2,-8, -9 and -10) and effector caspases (caspase-3, -6 and -7). The activation of caspase 3 will lead to

apoptosis and DNA fragmentation. Bax counteracts the antiapoptotic effects of Bcl-2. The translocation of Bax to mitochondria can alter the outer membrane permeability and activates the caspase cascade, leading to apoptotic death. Previous studies have demonstrated that IOA induces apoptosis in MCF-7, MDA-MB-231, Hep G2 and A549 cell lines through caspase-dependent or caspase-independent pathway [6-10]. IOA induced apoptosis associated with reduction in mitochondrial membrane potential and changed Bax/Bcl-2 ratio, caspase-9 activation, and cytochrome c release in MCF-7 and MDA-MB-231 cell line [6]. IOA also disrupts the function of mitochondrial, activates caspase-9, caspase-3, cleavage of PARP activaction and releases cytochrome C in A549 cells [10]. It is interesting that IOA induced apoptosis in caspase-dependent or caspase-independent pathway in Hep G2 cells [7-9]. One study reported that IOA changed Bax translocation to mitochondrial, resulted in release of cytochrome C and activated caspase-3 and PARP clevage in Hep G2 cells [7]. IOA also elicited the nuclear translocation of apoptosis-inducing factor (AIF) associated with large-scale DNA fragmentation independently of caspase recruitment [8].

Cell cycle

The cell growth and proliferation are controlled by cell cycle regulators. The cell cycle can divide into four phase including G_0/G_1, S, G_2, and M phase. Cyclins and cyclin-dependent kinases (CDK) control the cell cycle progression. P53 is a tumor suppressor gene and known to cause cell-cycle arrest or induce apoptosis. Many mediators can control p53-mediated cell cycle arrest. In MCF-7 and MDA-MB-231 breast cancer cells, IOA caused a significant inhibition of cycle progression in G_2/M phase, increased p21 expression, reduced cyclin expression including cyclin B1, cyclin A, cdc2 and cdc25 expression [6]. Moreover, IOA also increased the expression of inactive phospho-cdc2 and phospho-cdc25 in MCF-7 and MDA-MB-231 cells [6]. In A549 cells, IOA caused cell-cycle arrest in G0/G1 phase associated with increased the expression of p53, p21, and p27 [10].

Reactive oxygen species (ROS) generation and apoptosis

Mitochondria are considered as the main source of ROS. ROS are highly reactive oxygen free radicals generated by multiple mechanisms. ROS can regulate by a number of cellular pathways and play a dual role in determining the fate of cell survival and death [12].

IOA can generate the ROS production and this effect can be blocked with ROS scavenger (N-acetyl-L-cysteine) and NADPH inhibitor (diphenyleneiodonium chloride) in Hep G2 cells [7]. In addition, one study has shown the ROS production of IOA can be decreased by pretreating with either antioxidant or NO inhibitor such as N-acetyl-L-cysteine, catalase, mannitol, dexamethasone, trolox or L-NAME in A549 cells. Further, antioxidant (EUK8) or N-acetyl-L-cysteine can be completely bolcked ROS production induced by IOA in MCF-7 and MDA-MB-231 cells [6].

ASK1 is a member of the MAPK kinase kinase (MAPKKK) family and upstream activator of MAPK signaling cascades [13]. MAPK signal pathway also plays an important role in oxidative stress-induced apoptosis. Signal-regulating kinase 1 (ASK1)/mitogen-activated protein kinase (MAPK) signaling pathway seem to be mediated in part by activation of the apoptosis. Study has demonstrated that IOA activated ASK1 and induced the expression of c-Jun NH2-terminal kinase and p38 in the MCF-7 and MDA-MB-231 [6]. However, these effects can be blocked by antioxidant (EUK8) or N-acetyl-L-cysteine through inhibiting the ASK1 at Thr^{845} phosphorylation and dephosphorylation at Ser^{967}. These results implicate that the generation of ROS plays an important role in IOA-induced apoptosis in breast cancer [6].

Recently, tumor necrosis factor-related apoptosis-inducing factor ligand (TRAIL) is considered as a target for inducing apotosis. TRAIL is a protein and binds to death receptor 4 (DR4) or death receptor 5 (DR5) leading the formation of death-inducing signaling complex [14]. CCAATT/enhancer binding protein homologous protein (CHOP) is a protein involved in endoplasmin reticulum regulating apoptosis. CHOP also modulates DR5 expression via binding to a CHOP binding site and induces apoptosis. The report has shown that co-incubation of TRAIL and IOA significantly induces caspase-dependent apoptosis by up-regulation of C/EBP homologous protein and DR5 protein levels in Hep G2 cells [9]. Further, DR5 expression is associated with IOA treatment accompanied by provoking intracellular ROS generation [9].

Toxicological Study and *in vivo* Tumor Xenograft Study

The administration of IOA at doses of 350, or 700 mg/kg bw did not change body weight, the weight percentage of liver, spleen and kidney to the body weight without toxicity in Sprague-Dawley rats [7].

Kuo et al. has shown that IOA at doses of 4 mg/kg has significant cancer cell growth inhibition (MDA-MB-231 cells) by i.p. injected daily with IOA in nude mice. Moreover, increases of phospho-ASK1, phospho-JUK, phospho-p38, and TUNNEL-positive cells were observed in tumors of IOA-treated mice [6].

Table 1 The proposed target proteins and mechanisim of IOA on the inhibition of cancer in vitro and in vivo.

Cancer type	Cell/animal type	Biological effects	Molecular targets	Reference
Heptatoma	Hep G2	1. ↑:Reactive oxygen specis (ROS) 2. ↑:cell cycle sub-G1 fraction; 3. disruption of mitochondrial transmembranepotenial 4. apoptosis	1. ↑:NADPH oxidase 2. ↑: cleaved caspase; cleaved PARP, cytosol cytochrome C; mittochondrialBax 3. DNA fragmentation 4.↑:C/EBP homologus protein (CHOP); death receptor 5 (DR5)	7, 8, 9
Breat	1. MCF-7/MDA-MB-231 2. MDA-MB-231 xenograt mice	1. ↑:ROS 2. Cell cycle G2/M arrest 3. disruption of mitochondrial transmembranepotenial 4. apoptosis	1. ↑:p21; phosphorylated cdc2 and cdc25 2. ↑: Bax/Bcl-2 rations; caspase-9; cytosol cytochrome C 3. ↑:activated apoptosis signal regulating kinase 1 (AS1); c-Jun NH_2-terminal kinase; p38	6
Lung	A549	1. ↑:Reactive oxygen specis (ROS) 2. ↑:cell cycle sub-G1 fraction; 3. disruption of mitochondrial transmembranepotenial 4. apoptosis	1.↑:p21; P27 2. ↑: Bax/Bcl-2 rations; caspase-9; caspase-3; cytosol cytochrome C; cleaved PARP	5, 10

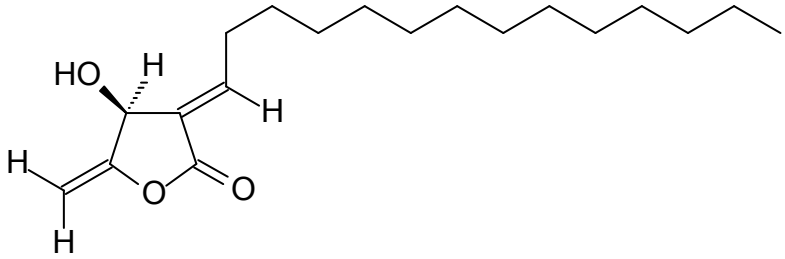

Figure 1 The chemical structure of IOA.

CONCLUSION

IOA multiple mechanisms of actions in inhibiting cancer cells growth are shown in table 1. In general, IOA inhibits cancer cells growth by mainly producing ROS and cell cycle arrest mediated apoptosis. Base on these studies, IOA could be as a chemotherapeutic agent. However, the more *in vivo* studies should be conducted.

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