Pentacyclic triterpenoic acids: new chemoprotective compounds*

Minireview

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Ursolic acid and oleanolic acid are pentacyclic triterpenoic acids having a similar chemical structure and are the major components of some oriental and traditional medicine herbs wildly distributed all over the world. There is a growing interest in the elucidation of the biological roles of both these triterpenoid compounds. This review summarizes the biological activities of presented triterpenoid acids (anti-inflammatory, hepatoprotective, gastroprotective, anti-ulcer, anti-HIV, cardiovascular, hypolipidemic, antiatherosclerotic and immunoregulatory effects). Our interest has been focussed especially on their anti-tumor and chemopreventive activity. Both compounds have been shown to act at various stages of tumor development, including inhibition of tumorigenesis, inhibition of tumor promotion, and induction of tumor cell differentiation. They effectively inhibit angiogenesis, invasion of tumor cells and metastasis. However, the mechanisms by which they act are poorly understood. Ursolic acid and oleanolic acid are relatively non-toxic and could be use as chemopreventive/chemoprotective agens in clinical praxis.

Key words: oleanolic acid, ursolic acid, anti-tumor, anti-inflammatory, immunomodulatory properties

In recent years, the development of more effective and safer agents has been intensively required for chemoprevention of human cancer, and natural products from plants and their synthetic derivatives have been expected to play an important role in creating new and better chemopreventive agents. In particular, there has been a need for chemopreventive agents targeted at the promoting stage of carcinogenesis in the two or multi-stage theory since it is difficult to avoid unfavorable hits by tumor-initiating agents in human life [54].

The aim of our review was to compare the biological effects of two pentacyclic triterpenoid acids: ursolic acid (UA) and oleanolic acid (OA). UA (3β-hydroxy-urs-12-en-28-oic acid) and its isomer, OA (3β-hydroxy-olea-12-en-28-oic acid), are triterpenoid acids which exist widely in plants in the form of free acids or aglycones for triterpenoid saponins [34]. They have a similar molecular structure, but have different sites of the methyl group on the E loop: if the methyl group at C_{19} of UA is moved to C_{20}, it becomes OA (Fig. 1) [32].

UA and OA are compounds naturally found in various plants such as sea-weeds, the wax-like coatings of fruits and many medicinal herbs, e.g. rosemary, thyme, oregano and lavender (Tab. 1). They were long considered to be biologically inactive but in recent years, they have attracted the interest of medical scientists because of their pharmacological effects, combined with a low toxicity [40]. The traditional uses of plants containing UA or OA in folk medicine are multiple, in terms of anti-inflammatory, hepatoprotection, analgesia, cardiotonic, sedative and tonic effects, etc. Many of these therapeutic effects have been confirmed by contemporary scientific research [34].

Anti-tumor and chemopreventive activity

Both tumor initiation and promotion are inhibited by UA and OA to various degrees. The most notable effect of these two triterpenoids is anti-tumor-promotion [34].

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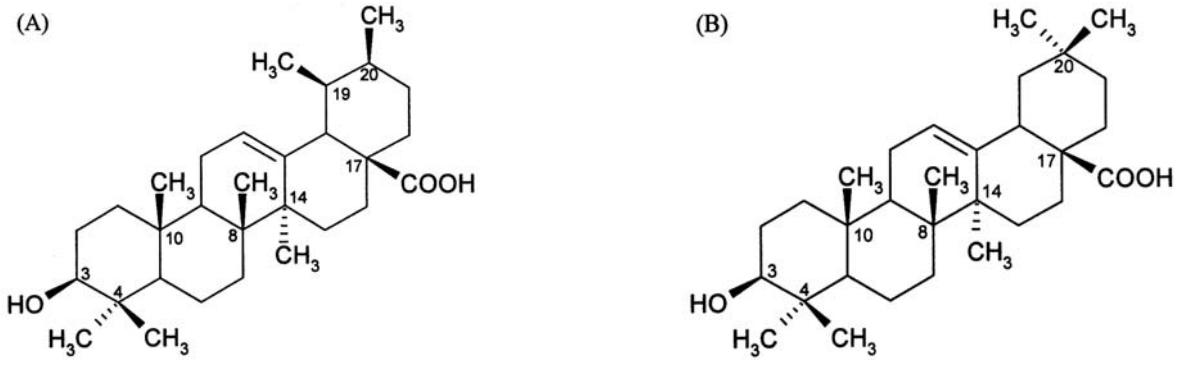

Figure 1. Chemical structures. (A) ursolic acid, (B) oleanolic acid.

Table 1. Occurrence of ursolic acid and oleanolic acid in folk medicine

Name	Latin name	Extracted from	Compound	Ref.
Rosemary	*Rosmarinus officinalis L.*	leaves	OA, UA	[1,21,49]
Elder berry	*Sambucus nigra, Sambucus Canadensis*		OA, UA	[22]
Periwinkle	*Vinca minor L.*	leaves	UA	[18,28]
Ginseng	*Panax ginseng* roots		OA	[34]
Oleander	*Nerium oleander L.*	leaves	UA	[28]
Sage	*Salvia officinalis L.*	leaves	UA	[28]
Oregano	*Origanum vulgare, Origanum majorana L.*		UA	[12,17,19]
Thyme	*Thymus broussonettii, Thymus willdenowii*	leaves	OA, UA	[23,24]
Blacthorn	*Prunus spinosa L.* f	lowers, leaves	OA, UA	[57]
Japanese cherry	*Prunus serrulata var. spontanea*	leaves	UA	[25]
Chinese hawthorn	*Crataegus pinatifida*	leaves	UA	[37,38]
Ground ivy	*Glechoma hederacea L.*		OA, UA	[41,55]
Glossy privet	*Ligustrum lucidum Ait*		OA, UA	[33]
Cynomorium herb	*Cynomorium songaricum*	stems	UA	[35]
Plantain	*Plantago major L.*	leave wax	OA, UA	[46]
Guava	*Psidium guajava*	leaves	OA, UA	[8]
Spurge	*Euphorbia portlandica, Euphorbia segetalis*		OA	[36]
Jujube	*Zizyphus jujube*	fruits	OA	[30,32]
Beefsteak plant	*Perilla frutescens*	leaves	UA, OA	[7,10]
Gardenia	*Gardenia saxatilis*	twigs	OA, UA	[52]
Madagascar periwinkle	*Catharantus roseus L.*		UA	[32]
Almond hulls			OA, UA	[53]
Arjuna	*Terminalia arjuna* root	bark	OA	[56]
Syzygium	*Syzygium buxifolium*	stem, root	UA, OA	[59]
Eucalyptus	*Eucalyptus hybrid E.tereticomis*	leaves	UA	[47]
Chinese bladdernut	*Staphylea holocarpa Hemsl.*		UA	[39]
Persimmon	*Diospyros leucomelas*		UA	[43]
Wild rose	*Rosa woodsii*	leaves	OA	[26]
Honey mesquite	*Prosopis glandulosa*	leaves, twigs	OA	[26]
Juniper mistletoe	*Phoradendron juniperinum*	whole plant	OA	[26]
Scotch heather	*Calluna vulgaris*	heater flowers	UA	[14]
Formoasan sweetgum	*Liquidambar formosana*	fruits	OA	[13]

UA and OA, which had been isolated from *Glechoma hederacea* as inhibitors of Epstein-Barr virus activation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), were tested against inhibitory effect on tumor promotion by TPA *in vivo*. They inhibited effectively the tumor promotion in mouse skin. Interestingly, UA was more effective on a single application before initial TPA-treatment than on a continuous application before each TPA-treatment, while OA was ineffective in the same treatment. These data suggest that the role of UA for inhibitory action on tumor promotion differs slightly from those of OA [55].

The anti-tumor-promoting potency of UA and OA can be understood from hypothesis that the protein kinase C signaling pathway would be involved in their inhibition [7].

HSU et al [20] investigated the exponentially growing of HL-60 cells with various concentrations of UA or OA, respectively, or combined with different dose of radiation. The cellular damage induced by UA and OA may be dis-

turbed by radiation. The more dose of radiation used the more inhibitory effect of both UA and OA on HL-60 cells can be found.

Apoptosis

KIM et al [27] investigated the apoptotic effect of UA on HepG2 human hepatoblastoma cells. Treatment with UA decreased the viability of HepG2 cells in a concentration- and time-dependent manner. Furthermore, 30 μM of UA induced DNA fragmentation and subdiploid cells and enhanced the release of cytochrome c and the activation of caspase-3. These results suggest that UA induces cell death through apoptosis, which may be mediated by cytochrome c-dependent caspase-3 activation.

ANDERSSON et al [3] studied the anticancer effect of UA on human colon cancer cells HT-29. UA dose-dependently decreased cell proliferation and induced apoptosis, accompanied by activation of caspase 3, 8 and 9. UA selectively increased the activity of intestinal alkaline sphingomyelinase, which occurred before activation of caspases. UA had no effect on alkaline phosphatase activity. These results suggested that UA has strong anti-proliferative and apoptotic effects on HT-29 cells. The effects may be mediated by alkaline sphingomyelinase activation.

LEE et al [29] prooved the anti-tumor effect of UA and UA-induced metabolic changes in tumors growing in mice. They evaluated MTT colorimetric assay, clonogenic assay, and growth-delay assay for the determination of tumoricidal effects of UA. UA-induced apoptosis was measured by fluorescent microscopy, stained by propidium iodide. Oxygen consumption after treatment with UA was measured using a Clark-type electrode chamber. UA induced apoptosis significantly increased in a dose-dependent manner. Cellular oxygen consumption values were significantly reduced by UA. In animal studies, UA significantly reduced tumor interstitial fluid pressure to 40% of the control values at 2-3 days post-treatment. An i.p. administration of 100 mg/kg of UA significantly inhibited tumor growth of FSaII. In conclusion, UA showed anti-tumor effect on various tumor cells *in vitro* (CCL-209, FSaII, MCaIV, BT-20, U87, AsPC-1, A549) as well as a moderate retardation of growth in two tumor models *in vivo* (MABP, TIFP). In general, murine tumor cells were more sensitive to UA than human tumor cells.

OA inhibited the growth and induced apoptosis of K562, an erythroleukemia cell line. Most importantly, it also inhibited the proliferation of Lucena 1, a vincristine-resistant derivative of K562 that displays several multidrug resistance (MDR) characteristics. Taken together, OA is a potent anti-MDR agent [15].

UA inhibits HL-60 leukemic cell proliferation and arachidonic acid oxidative metabolism in various cell lines. OA, which differs by the position of one methyl group (C_{20} instead of C_{19}) is less inhibitory than UA [14].

Antioxidant activity

Antioxidant activity of UA isolated from *Ocimum sanctum* and OA from *Eugenia jumbolana* against free radical induced damage was studied by BALANEHRU et al [6]. They have used three different standard systems viz., ascorbic acid, carbon tetrachloride and ADP/Iron to induce lipid peroxidation in isolated rat liver microsomes *in vitro*. Both OA and UA offered remarkable protection of 90% and 60%, respectively. Both compounds did not induce lipid peroxidation by themselves that improved the therapeutic application. Later BALANEHRU et al [5] studied the free radical scavenging potential of these compounds against adriamycin induced lipid peroxidation both in liver and heart microsomes *in vitro*. They have identified OA as a strong protector against adriamycin induced lipid peroxidation and UA as a mild protector. Protection with OA was 49% and 21% in liver and heart microsomes, respectively. UA showed only 13% and 17% protection in liver and heart microsomes.

Amyloid beta protein (Abeta) increases free radical production and lipid peroxidation in PC12 nerve cells, leading to apoptosis and cell death. HEO et al [19] investigated the effect of UA from *Origanum majorana L.* on Abeta-induced neurotoxicity using PC12 cells. Pretreatment with isolated UA and vitamin E prevented the PC12 cell from reactive oxygen species toxicity that is mediated by Abeta. Their results indicate that micromolar Abeta-induced oxidative cell death is reduced by UA from *Origanum majorana L.*

Cytotoxicity

MIN et al [38] tested the cytotoxic activity of UA against murine L1210 and human cancer cell lines (A549, SK-OV-3, SK-MEL-2, XF498, and HCT15) *in vitro*. UA showed moderate cytotoxicity against L1210, whereas it showed weak activity against human cancer cell lines.

UA and OA possessed strong activity against human leukemia and lymphoma cell lines. UA was effective against Burkitt lymphoma cells P3HR1 (IC_{50} 2.5 μg/ml, SI 262.6) and chronic myelogenous leukemia cells K562 (IC_{50} 17.79 μg/ml, SI 36.91), whereas OA inhibited the growth of P3HR1 cells (IC_{50} 26.74 μg/ml, SI 11.37) [11].

LI et al [32] investigated the inhibitory effects of UA and OA on human colon carcinoma cell line HCT15. The cytotoxicity of UA was stronger than that of OA. The IC_{50} values for UA and OA were 30 and 60 μmol/l, respectively. Proliferation assay showed that cell viability was signifi-

cantly decreased in a concentration-dependent manner and proliferation was markedly inhibited by both drugs. It was shown that both drugs possessed an inhibitory effect on HCT15 cells. According to changes in HCT15 cell morphology, UA and OA have a direct cytotoxic effect on HCT15 cells. When treated with IC_{50} concentration for 36 and 72 hours, the G_0/G_1 phase cells were gradually increased, with a concomitant decrease of cell population in S phase and no detectable apoptotic fraction.

In an acute toxicity test on mice, intraperitoneal administration of OA showed no toxicity at doses up to 600 mg/kg [4].

RODRIGUEZ et al [45] determined that OA improves healing of chronic gastric lesions in rats. The low toxicity and widespread occurrence of OA suggest it as a potential new antiulcer drug.

Anti-angiogenic activity

Angiogenesis is important for the progressive growth of solid tumors and even for capacity of metastatic spread from tumor primary site. UA and OA were examined for anti-angiogenic activities by using the chick embryo chorioallantoic membrane (CAM) assay [50]. The presence of UA or OA inhibited angiogenesis in a dose-dependent manner; the doses required for half-maximal inhibition (ID_{50}) were 5 μg and 40 μg per CAM, respectively. UA was a more potent angiogenic inhibitor than OA. UA and OA effectively inhibited even the proliferation of bovine aortic endothelial cells in a concentration-dependent manner. Based on these results, authors speculated that the inhibitory effects on bovine aortic endothelial cell proliferation of UA and OA might be important for anti-angiogenesis [50].

Effects of UA and OA on the molecular level

The process of tumorigenesis requires cellular transformation, hyperproliferation, invasion, angiogenesis, and metastasis. Several genes that mediate these processes are regulated by the transcription factor nuclear factor-κB (NF-κB). The latter is activated by various carcinogens, inflammatory agents, and tumor promoters. Thus, agents that can suppress NF-κB activation have the potential to suppress carcinogenesis. UA has been shown to suppress the expression of several genes associated with tumorigenesis. UA also suppressed NF-κB activation induced by various carcinogens including tumor necrosis factor-α (TNF-α), phorbol ester, okadaic acid, H_2O_2, and cigarette smoke. These effects were not cell type specific. UA inhibited DNA binding of NF-κB consisting of p50 and p65. UA inhibited IκBα degradation, IκBα phosphorylation, IκBα kinase activation, p65 phosphorylation, p65 nuclear translocation, and NF-κB-dependent reporter gene expression. UA also inhibited NF-κB-dependent reporter gene expression activated by TNF receptor, TNF receptor-associated death domain, TNF receptor-associated factor, NF-κB-inducing kinase, IκBα kinase, and p65. The inhibition of NF-κB activation correlated with suppression of NF-κB-dependent cyclin D1, cyclooxygenase 2, and matrix metalloproteinase 9 expressions. Thus, UA inhibits IκBα kinase and p65 phosphorylation, leading to the suppression of NF-κB activation induced by various carcinogens. These actions of UA may mediate its anti-tumorigenic and chemosensitizing effects [48].

To determine the mechanism underlying anti-tumorigenic effects of UA YOU et al [58] investigated the effects of UA in the release of nitric oxide (NO) and TNF-α, and on the level of inducible nitric oxide synthase (iNOS) and TNF-α gene expression in mouse resting macrophages. They found that UA elicited a dose-dependent increase in NO and TNF-α production, and the level of iNOS and TNF-α mRNA. Transient expression and electrophoretic mobility shift assays with NF-κB binding sites revealed that the increased level of iNOS mRNA and TNF-α mRNA induced by UA were mediated by the NF-κB transcription factor complex. These results demonstrate that UA stimulates NO and TNF-α release and is able to upregulate iNOS and TNF-α expression through NF-κB transactivation in the resting macrophages.

UA may be an effective inhibitor of UVA-modulated signal transduction pathways in human skin cells. UA significantly suppressed the UVA-induced reactive oxygen species production and lipid peroxidation. Pretreatment with UA significantly reduced the UVA-induced activation and expression of matrix metalloproteinase-2. UVA-induced enhanced expression of p53, a hallmark of UV-induced DNA damage and cell death, was also significantly inhibited by pretreatment with UA [31].

OA significantly inhibited TNF-α-induced expression of intercellular adhesion molecule (ICAM-1) on human monocytic leukemia cells THP-1 [2]. This compound had a weak inhibitory activity on the "nuclear factor of activated T cells" with IC_{50} value over 50 μM [13].

UA showed a significant cyclooxygenase 2 inhibitory effect, directly on the enzyme activity. The structural isomer OA was found to be less active than UA [44].

Anti-inflammatory effects

The inhibitory effect of UA and OA on TPA-induced inflammation has been demonstrated to closely parallel that of the inhibition of tumor promotion in two-stage carcinogenesis initiated by 7,12-dimethylbenz[a]anthracene (DMBA) and then by TPA, a well-known promoter, in a mouse skin model. UA and OA markedly inhibited the

TPA-induced inflammation at 0.10 and 0.30 mg/ear of 50% inhibitory dose [7].

In order to determine whether further oxidation of carbon 3 affects anti-inflammatory activity in mice, different tests were carried out on OA and its 3-oxo-analogue oleanonic acid. The presence of a ketone at C-3 implies an increase in the inhibitory effect on models related to 5-lipoxygenase activity and on associated *in vivo* inflammatory processes [16].

UA showed potent considerable effect on Epstein-Barr virus early antigen induction [7] and ISMAILI et al [23, 24] identified UA and OA as the main compounds responsible for the anti-inflammatory activity of Moroccan traditional medicinal herbs *Thymus broussonetii* and *Thymus willdenowii.*

Hepatoprotective and gastroprotective effects

UA showed the hepatoprotective activity against a wide range of chemically induced hepatic damage. Antagonism of the toxic effects of galactosamine, thioacetamide and carbon tetrachloride indicates that UA might protect the liver by increasing protein synthesis, it could stabilize the cell membrane and inhibit the toxin including free radical generation and then act as a potent hepatoprotective agent [47].

OA shows gastroprotective effects in ethanol, aspirin and pilorous ligature-induced gastric ulcer in rats as well as in the ethanol/hydrochloric acid-induced ulcer in mice. OA improves healing of chronic gastric lesions in rats. The mechanism of gastroprotective effect of OA is not yet clear [45].

Other effects

Many biological activities of UA and OA, including anti-HIV effect, inhibition of DNA and polymerase β, HIV-1 protease, chitin synthetase 2, inhibition of TPA activity and insoluble glucan synthesis, are responsible for anti-inflammatory and anti-hyperlipidemic properties as well as for anti-tumor-promotion effects. UA and OA also display anti-piretic and analgesic activity [45].

RAPHAEL et al [42] studied the effect of UA and OA on the immune system using Balb/c mice. In UA and OA treated animals the percentage of increase in the total white blood cells count was to 91.48% and 135.75%, respectively. The immunomodulatory activity of studied triterpenoid acids remarkably produced an enhancement of specific antibodies and together they inhibited hypersensitivity reaction.

UA inhibited HIV-I protease with IC_{50} value of 8.0 μmol/l [37]. OA inhibited HIV-1 replication in acutely infected human T cells H9 with EC_{50} value of 1.7 μg/ml, and inhibited H9 cell growth with an IC_{50} value of 21.8 μg/ml. UA did show anti-HIV activity (EC_{50} 2.0 μg/ml), it was slightly toxic (IC_{50} 6.5 μg/ml) [26].

SOMOVA et al [51] studied the cardiovascular (systolic and diastolic blood pressure, heart rate), antihyperlipidemic (triglycerides, total cholesterol and lipoprotein fractions), antioxidant (glutathione peroxidase – GPx, and superoxide dismutase – SOD), diuretic/saluretic and hypoglycemic activity of 98% pure OA and UA in Dahl salt-sensitive (DSS), insulin resistant rat model of genetic hypertension. Both OA and UA displayed low toxicity, with LC_{50} 0.10 and 0.95 mg/ml, respectively. Although both triterpenoid acids did not have direct hypotensive effect, after 6-week application in a daily dose of 60 mg/kg b.w., i.p., they prevented the development of severe hypertension. The antihypertensive effect was attributed to their considerable diuretic-natriuretic-saluretic activity; direct cardiac effect (heart rate decrease by 34% and 32%, respectively); antihyperlipidemic (more than two times decrease of LDL and triglycerides) as well as antioxidant (GPx increase by 12% and 10%, respectively; SOD increase 12% and 22%, respectively) effect on the DSS rats.

UA appeared to be a potent acetylcholine inhibitor in Alzheimer disease. Acetylcholinesterase inhibitors, which enhance cholinergic transmission by reducing the enzymatic degradation of acetylcholine, are the only source of compound currently approved for the treatment of Alzheimer's disease [12].

BOTH et al [9] demonstrated that UA incorporated into liposomes (URA liposomes) increases both the ceramide content of cultured normal human epidermal keratinocytes, and the collagen content of cultured normal human dermal fibroblasts.

Conclusion

Water insoluble compounds UA and OA possess various anti-tumor activities, such as inhibition of tumorigenesis, tumor promotion as well as induction of tumor cells differentiation. Both compounds have been shown to inhibit effectively angiogenesis and invasion, which are importantly involved in the process of tumor metastasis. They may be therefore effective anti-cancer agents acting at various stages of tumor development. They possessed strong activity against human leukemia and lymphoma cell lines. In experimental models UA inhibited the growth of leukemia, melanoma, hepatoma and carcinomas of lung, ovary, colon, cervix and brain, and OA was active against leukemia and carcinomas of lung, breast, pancreas, colon and kidney. OA has been shown to be a new potent anti-MDR agent.

The toxicity of UA and OA is low and their distribution in plants is extensive. Besides their anti-tumor activity, they

also possess anti-inflammatory, hepatoprotective, antiulcer, hypolipidemic, antiatherosclerotic and immunoregulatory effects, anti-HIV activity by inhibiting dimerization of HIV-1 protease.

Therefore, they have a bright future in clinical application. Further investigation to explore their potential use in clinical situation may prove to be worthwhile.

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