Review

Biological activities of sinularin: A literature-based review

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Abstract: Sinularin ((9E)-13-hydroxy-4,9,13-trimethyl-17-methylidene-5,15-dioxatricyclo[12.3.1.0(4,6)] octadec-9-en-16-one) is the soft coral-derived hopeful biologically active lead compound. In this review sinularin biological activities are summarized. For that, an up-to-date (from 1980 to Mar 2020) search was made in the PubMed, Science Direct, Web of Science, Scopus, The American Chemical Society, Clinicaltrials.gov, and Google Scholar databases. Data available suggest that sinularin has interesting anti-inflammatory, anticancer, anti-fouling and analgesic potential. The inducible nitric oxide synthase (iNOS), cyclooxigenase (COX)-2, tumor growth factor beta 1 (TGF-β1) are the most efficient enzymes for interacting with sinularin due to its anti-inflammatory activity, while phosphoinositol 3-kinase (PI3K), Akt and mechanistic target of rapamycin (mTOR) for its anticancer effect. In conclusion, sinularin seems to be a promissory lead compound in the treatment of inflammation, cancer and neurological disorders.

Key words: Marine drugs; Sinularin; Inflammation; Cancer.

Introduction

The oceans are home to around 90% of the world's living biomass, therefore, the marine environment is an extraordinary and rich reservoir of bioactive natural products with multiple pharmacological potentialities (1). Sinularin is a natural compound isolated from marine soft corals (e.g., *Sinularia triangular*, *S. querciformis*, *S. flexibilis*), and increasing evidences have suggest that it possess some important biological effects, including anti-inflammatory and analgesic, anti-fouling and anticancer effects (2,3,8,9). Nonetheless, although it has been isolated and identified 30 years ago, scientific reports regarding its biological effects are not enough.

In this sense, the present review aims to sketch the current scenario on sinularin biological effects based on scientific reports from various databases.

Methods

An up-to-date (till Mar 2020) search was made in the PubMed/MedLine, Science Direct, Web of Science, clinicaltrials.gov, and Google Scholar databases, selecting as main keyword "sinularin", which was then paired with the following keywords "biological sources", "biological effects", "pharmacological activity", and "toxicology". Scientific reports were then target of a strict scrutiny and analyzed considering the following inclusion and exclusion criteria.

Inclusion criteria

1. Studies carried out *in vitro, ex vivo, in vivo, in silico* with or without using experimental animals, including humans and their derived tissue and cells;

2. Studies with sinularin and its derivatives or preparations;

3. Studies with or without proposing activity mechanisms;

4. Studies regard to any biological/pharmacological activity.

Exclusion criteria

1. Duplication of data and titles and/or abstracts (in the databases) or not meeting the inclusion criteria; and

2. Studies on crude coral extract having no information on sinularin presence/determination.

After application of the above-mentioned filters, a total of 13 scientific reports were found, and selected to be discussed here.

Biological effects of sinularin

Over the years, several biological activities have been proposed and increasingly confirmed to sinularin, namely with concerns to its anti-inflammatory, analgesic, anticancer, and anti-fouling effects (Table 1).

Anti-inflammatory effect

Sinularin isolated from *Sinularia triangular* have revealed interesting anti-inflammatory effects (3). For example, orally administered sinularin reduced carrageenan-induced paw edema 3 h after carrageenan injection, and after adjuvant administration inhibited adjuvant-induced paw swelling (periarthritis model) over a 21-day period (11). In another study, sinularin isolated from *Sinularia querciformis* significantly inhibited proinflammatory proteins upregulation, particularly inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in lipopolysaccharide (LPS)-stimulated murine macrophage cells (12).

Analgesic effect

Sinularin at 20 and 80 mg/kg (s.c.) in rats (n=6), and at 0.1-20 µM in RAW 264.7 cells was found to exert analgesic effects, possibly through leukocyte infiltration inhibition and transforming growth factor beta 1 (TGF-β1) upregulation (2).

Anti-cancer effect

Sinularin isolated from the soft coral *Sinularia flexibilis* has been reported to have anticancer activity against human epidermoid carcinoma (ED$_{50}$: 0.3 µg/mL)

and murine P388 lymphocytic leukemia (ED$_{50}$: 0.3 µg/mL) cell lines (13). Sinularin (1-5 µM) was also able to cause apoptotic cell death through overexpressing p53, caspase-3, -8, -9, p21, and Bax, while downregulated Bcl-2 expression in A2058 melanoma cells (3). Lei et al. demonstrated that sinularin exerted cytotoxic effects in HepG2, HepG2/ADM, MCF-7, and MCF-7/ADM cell lines, with IC$_{50}$ values being >50 µM (4).

Sinularin (3-18 µM) was also able to triggers apoptotic cell death through mitochondrial membrane potential loss, cytochrome *C* release, Bax, Bad and caspase-3/9 activation, and p-Bad, Bcl-xL and Bcl-2 suppression in human gastric cancer (AGS and NCI-N87) cell lines (5). In this study, an inhibition of the phosphoinositol 3-kinase (PI3K)/Akt/mechanistic target of the rapamycin (mTOR) signaling pathway was also seen. Moreover, sinularin (7.5, 15, 30, 60 µM) exerted reactive oxygen species (ROS)-mediated anti-proliferation, cdc2 and cyclin B1 mediated G2/M phase arrest; caspases-3, -8, -9 and poly (ADP-ribose) polymerase (PARP) activation and induced apoptosis in Ca9-22, CAL 27, HSC-3 and HGF-1 cells (6). Sinularin (25, 50, 75, 100 µM) also triggered DNA damage and cell cycle arrest at G2/M along with p-ATM (Ser(1981)), p-Chk2 (Tyr(68)), p-cdc2 (Tyr(15)), and p53 up-regulation, coupled with the increase in p21 expression and p-cdc25 (Ser(216)) down-regulation in HepG2 and Hep3B cell lines (7). In this study, sinularin also led to apoptotic cell death through decreasing Bcl-2 expression, inducing mitochondrial membrane potential disruption, and sequential activation of caspases and PARP. Moreo-

Table 1. Sinularin biological activities and proposed mechanisms of action.

Dose, Administration route and test system	Biological activity (Proposed mechanism of action)	References
20 and 80 mg/kg (s.c.) in rats (n = 6) & 0.1-20 µM in RAW 264.7 cells	Analgesic effects; inhibition of leukocyte infiltration and upregulation of TGF-β1	(2)
1-5 µM on A2058 melanoma cells	Apoptotic cell death (↑expression of p53; cleaved-caspase-3, cleaved-caspase-8, cleaved-caspase-9, p21, and Bax and ↓ expression of Bcl-2)	(3)
HepG2, HepG2/ADM, MCF-7, and MCF-7/ADM cell lines	Cytotoxic effects (IC$_{50}$: >50 µM)	(4)
3-18 µM on human gastric cancer (AGS and NCI-N87) cell lines	Apoptotic cell death (mitochondrial membrane potential loss, cytochrome *C* release, Bax, Bad and caspase-3/9 activation, and p-Bad, Bcl-xL and Bcl-2 suppression; inhibition of the phosphoinositol 3-kinase/Akt/ mechanistic target of the rapamycin signaling pathway)	(5)
7.5, 15, 30, 60 µM on Ca9-22, CAL 27, HSC-3 and HGF-1	ROS-mediated anti-proliferation; cdc2 and cyclin B1 mediated G2/M phase arrest; caspases 3, 8, 9 and poly (ADP-ribose) polymerase (PARP)	(6)
25, 50, 75, 100 µM on HepG2 and Hep3B cell lines	Triggered DNA damage; cell cycle arrest at G2/M associated with up-regulation of p-ATM (Ser(1981)), p-Chk2 (Tyr(68)), p-cdc2 (Tyr(15)), and p53 coupled with the increase in expression of p21 and down-regulation of p-cdc25 (Ser(216)); apoptosis characterized by decrease on Bcl-2 expression, mitochondrial membrane potential disruption, and sequential activation of caspases and PARP.	(7)
10, 50, 100 µg/mL in *Bugula neritina* and *Balanus albicostatus*	Anti-fouling activity (EC$_{50}$: >100 µg/mL)	(8)
20, 40, 60 µM on breast cancer (SKBR3 and MDA-MB-231) cells	Oxidative DNA damage; apoptosis PARP, caspases 3, 8, and 9 dependent pathways) and cell cycle arrest at G2/M	(9)
5-80 µM on human renal cancer cells 786-O and ACHN	caspase-3/-9 activation, mitochondrial proteins release, pro-apoptotic Bcl-2 family proteins up-regulation and anti-apoptotic Bcl-2 family proteins inhibition; PI3K/Akt/mTOR signaling pathway downregulation; MAPKs and p38 signaling activation.	(10)

ver, sinularin (7.5, 15, 30, 60 μM) also caused oxidative DNA damage and cell cycle arrest at G2/M phase and resulted in apoptotic cell death through PARP, caspases-3, -8, and -9-dependent pathways in breast cancer (SKBR3 and MDA-MB-231) cell lines (9). Sinularin (5-80 μM) was also able to activate caspase-3/-9, release mitochondrial proteins, up-regulate pro-apoptotic Bcl-2 family proteins and inhibit anti-apoptotic Bcl-2 family proteins (10). In this study, it was also found to downregulate PI3K/Akt/mTOR signaling pathway, while upregulate MAPKs and p38 signaling pathways in human renal (786-O and ACHN) cancer cells.

Anti-fouling effect

In a study, sinularin (10, 50, 100 μg/mL) was found to act against *Bugula neritina* and *Balanus albicostatus* (EC$_{50}$: >100 μg/mL) (8).

Discussion

Inflammation is part of the complex biological response of body tissues to harmful stimuli, including pathogens (e.g., bacteria, virus), damaged cells, or even irritants. It is a protective response governed by immune cells, blood vessels, and molecular mediators. Generally, this process eliminates the initial cause of cell injury, clear out necrotic cells and damaged tissues from the original insult and other inflammatory processes, and initiate tissue repair (14). However, it is evident that human beings' aging is characterized by a chronic, low-grade inflammation, better to be termed as "inflammaging", a highly pronounced risk factor for both morbidity and mortality in the elderly population due to inflammatory pathogenesis (15).

Nitric oxide synthases (NOS) are a family of isoforms responsible for the synthesis of the potent dilator nitric oxide (NO). Generally, iNOS expression occurs in inflammatory conditions, and produces large amounts of NO, which is one of the leading causes of cardiovascular diseases, such as atherosclerosis (16). On the other hand, COX-2 is a key enzyme in fatty acid metabolism, that is upregulated during both inflammation and cancer. It is induced by pro-inflammatory cytokines at the site of inflammation and enhances the synthesis of prostaglandins, stimulates cancer cell proliferation, promotes angiogenesis, inhibits apoptosis, and increases the metastatic potential. Therefore, COX-2 inhibitors are target of intense research interest toward potential clinical applications (17).

LPS acts as the prototypical endotoxin because it binds the CD14/TLR4/MD2 receptor complex in many cell types, such as monocytes, dendritic cells, macrophages and B cells, promoting the secretion of many inflammatory mediators, among them pro-inflammatory cytokines and NO (18). In a study, sinularin has shown to be able to inhibit the upregulated pro-inflammatory proteins, including iNOS and COX-2 in LPS-stimulated murine macrophage cells (12).

The interplay between nerve growth factor (NGF) and pain perception or other nervous system-related functions may directly influence growth factors and cytokines, such as tumor necrosis factor (TNF)-α and TGF-β1 in many cells, including human, murine, and bovine chondrocytes (19,20). This induction of NGF by

TGF-β1 is more potent than certain types of interleukins (ILs) stimulus, such as IL-1β (20). Sinularin also exerts analgesic effects in rats and RAW 264.7 cells by downregulating TGF-β1 expression (2).

Cancer constitutes a huge society burden in more and less economically developed countries worldwide (21). It has been reported that, the occurrence of cancer is increasing day-by-day (22). It is due to the population growth and aging, along with a raising prevalence of established risk factors (e.g., smoking, overweight, physical inactivity) and changing reproductive patterns associated with urbanization and economic development (23). Accumulated reports have demonstrated that natural products possess effective anti-cancer effects and may serve as alternative tools for cancer treatment (24). Approximately 50 years since the cytarabine (a marine-derived anticancer drug) approval, 4 approved drugs and 18 agents were target of clinical trials, 6 of which are in late development. However, in the recent decade, the discovery and development of anti-cancer drugs from marine origin has gaining much attention (25). According to the scientific evidence, sinularin was found to act against a number of cancer cell lines through distinct pathways, thus suggesting its promising and multi-edged sword-like anti-cancer effects. The possible anti-cancer pathways of sinularin in cultured cancer cells are briefly pictured in Figure 1.

Conclusion

Taken together, data related to sinularin bioactive effects markedly point out its prominent anti-inflammatory, anticancer, anti-fouling and analgesic potentialities. Its anti-inflammatory effects are due to iNOS, COX enzymes (especially COX-2) and TGF-β1 inhibition, while the anti-cancer activity have been proposed to be promoted through interaction with PI3K, Akt and mTOR-dependent pathways. Of note, sinularin may be one of the hopeful marine-derived therapeutic tools in the treatment of inflammation, cancer and neurological
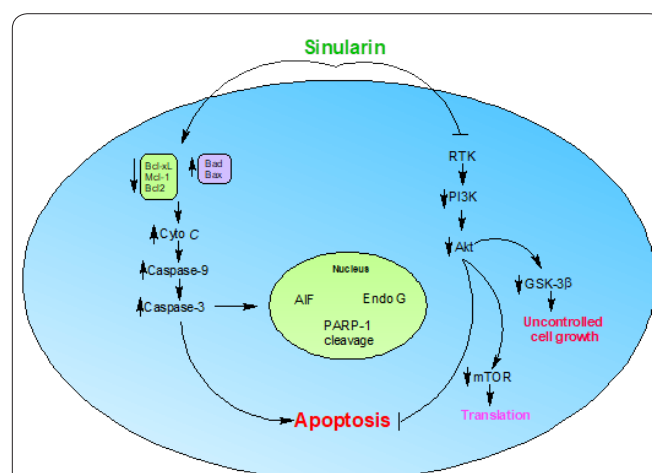
Figure 1. Anticancer effects of sinularin in cultured cancer cells. [Sinularin, through upregulation of Bad, Bax and downregulation of BcL-xL, Mcl-1 and Bcl-2, thereby up-regulates the expression of Cyto C and Caspase9/3 causes an apoptotic cell death. On the other hand, it can inhibit an uncontrolled cell growth through RTK/PI3K/Akt-GSK-3β-dependent pathway. The RTK/PI3K/Akt pathway is also responsible for its mTOR-dependent inhibition of translation in cancer cell proteins and an inhibition of apoptosis event in normal cells.]

diseases. Further studies are necessary to assess other sinularin-related pharmacological and toxicological effects in animal models.

Conflict of interest
The authors declare no conflict of interests.

References

1. Victor SP, Sharma CP. Anti-inflammatory drug delivery systems using marine products. Funct Mar Biomaterials 2015; 2015:137-47.

2. Huang SY, Chen NF, Chen WF, Hung HC, Lee HP, Lin YY, Wang HM, Sung PJ, Sheu JH, Wen ZH. Sinularin from indigenous soft coral attenuates nociceptive responses and spinal neuroinflammation in carrageenan-induced inflammatory rat model. Mar Drugs 2012; 10:1899- 919.

3. Su TR, Lin JJ, Chiu CC, Chen JY, Su JH, Cheng ZJ, Hwang WI, Huang HH, Wu YJ. Proteomic investigation of anti-tumor activities exerted by sinularin against A2058 melanoma cells. Electrophoresis 2012; 33:1139-52.

4. Lei L-F, Chen M-F, Wang T, He X-X, Liu B-X, Deng Y, Chen X-J, Li Y-T, Guan S-Y, Yao J-H, Li W, Ye W-C, Zhang D-M, Zhang C-X. Novel cytotoxic nine-membered macrocyclic polysulfur cembranoid lactones from the soft coral Sinularia sp. Tetrahedron 2014; 70:6851-8.

5. Wu YJ, Wong BS, Yea SH, Lu CI, Weng SH. Sinularin Induces Apoptosis through Mitochondria Dysfunction and Inactivation of the pI3K/Akt/mTOR Pathway in Gastric Carcinoma Cells. Mar Drugs 2016; 14(8). doi: 10.3390/md14080142.

6. Chang YT, Wu CY, Tang JY, Huang CY, Liaw CC, Wu SH, Sheu JH, Chang HW. Sinularin induces oxidative stress-mediated G2/M arrest and apoptosis in oral cancer cells. Environ Toxicol 2017; 32:2124-32.

7. Chung TW, Lin SC, Su JH, Chen YK, Lin CC, Chan HL. Sinularin induces DNA damage, G2/M phase arrest, and apoptosis in human hepatocellular carcinoma cells. BMC Complement Altern Med 2017; 17:62.

8. Wang J, Su P, Gu Q, Li WD, Guo JL, Qiao W, Feng DQ, Tang SA. Antifouling activity against bryozoan and barnacle by cembrane diterpenes from the soft coral Sinularia flexibilis. Int Biodet Biodegrad 2017; 120:97-103.

9. Huang HW, Tang JY, Ou-Yang F, Wang HR, Guan PY, Huang CY, Chen CY, Hou MF, Sheu JH, Chang HW. Sinularin Selectively Kills Breast Cancer Cells Showing G2/M Arrest, Apoptosis, and Oxidative DNA Damage. Molecules 2018; 23(4). doi: 10.3390/molecules23040849.

10. Ma Q, Meng X-Y, Wu K-R, Cao J-Z, Yu R, Yan Z-J. Sinularin exerts anti-tumor effects against human renal cancer cells relies on the generation of ROS. J Cancer 2019; 10:5114-23.

11. Buckle PJ, Baldo BA, Taylor KM. The anti-inflammatory activity of marine natural products – 6-n-tridecylsalicylic acid, flexibilide and dendalone 3-hydroxybutyrate. Agents Actions 1980; 10:361-7.

12. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, Currie MG. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol 1996; 118:829-38.

13. Weinheimer AJ, Matson JA, Hossain MB, van der Helm D. Marine anticancer agents: sinularin and dihydrosinularin, new cembranolides from the soft coral, Sinularia flexibilis. Tetrahedron Lett 1977; 18:2923-6.

14. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. Clin Exp Immunol 2007; 147(2):227-35.

15. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A: Biol Sci Med Sci 2014; 69(1):S4-S9.

16. Lind M, Hayes A, Caprnda M, Petrovic D, Rodrigo L, Kruzliak P, Zulli A. Inducible nitric oxide synthase: Good or bad? Biomed Pharmacother 2017; 93:370-5.

17. Desai SJ, Prickril B, Rasooly A. Mechanisms of Phytonutrient Modulation of Cyclooxygenase-2 (COX-2) and Inflammation Related to Cancer. Nutr Cancer 2018; 70(3):50-375.

18. Abbas A. Basic Immunology. Elsevier, 2006. ISBN: 978-1-4160-2974-8.

19. Lai Y, Bai X, Zhao Y, Tian Q, Liu B, Lin EA, Chen Y, Lee B, Appleton CT, Beier F, Yu XP, Li CJ. ADAMTS-7 forms a positive feedback loop with TNF-alpha in the pathogenesis of osteoarthritis. Ann Rheum Dis 2014; 73(8):1575-84.

20. Blaney Davidson EN, van Caam AP, Vitters EL, Bennink MB, Thijssen E, van den Berg WB, Koenders MI, van Lent PL, van de Loo FA, van der Kraan PM. TGF-β is a potent inducer of Nerve Growth Factor in articular cartilage via the ALK5-Smad2/3 pathway. Potential role in OA related pain? Osteoarthritis Cartilage 2015; 23(3):478-86.

21. Li Z, Aninditha T, Griene B, Francis J, Renato P, Serrie A, Umareddy I, Boisseau S, Hadjiat Y. Burden of cancer pain in developing countries: a narrative literature review. Clinicoecon Outcomes Res 2018; 10:675-91.

22. Shankar A, Saini D, Dubey A, Roy S, Bharati SJ, Singh N, Khanna M, Prasad CP, Singh M, Kumar S, Sirohi B, Seth T, Rinki M, Mohan A, Guleria R, Rath GK. Feasibility of lung cancer screening in developing countries: challenges, opportunities and way forward. Transl Lung Cancer Res 2019; 8(Suppl 1):S106-21.

23. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA. Cancer J Clin 2015; 65(2):87-108.

24. Huang XM, Yang ZJ, Xie Q, Zhang ZK, Zhang H, Ma JY. Natural products for treating colorectal cancer: A mechanistic review. Biomed Pharmacother 2019; 117:109142.

25. Pereira RB, Evdokimov NM, Lefranc F, Valentão P, Kornienko A, Pereira DM, Andrade PB, Gomes NGM. Marine-Derived Anticancer Agents: Clinical Benefits, Innovative Mechanisms, and New Targets. Mar Drugs 2019; 17(6):329.