NEWSLETTER N°4
July 2022

IUPHAR Mediterranean Group of Natural Products Pharmacology

Orobanche crenata Forssk. Extract Affects Human Breast Cancer Cell MCF-7 Survival and Viral Replication

COMMENTED BY Francesco Margiotta, *Department of Neuroscience, Psychology, Drug Research and Child Health - NEUROFARBA - Pharmacology and Toxicology Section, University of Florence, Viale G. Pieraccini 6, 50139 (Florence), Italy*

AUTHORS Carlo Genovese[1,2], Adriana Garozzo[3], Floriana D'Angeli[4], Giuseppe Antonio Malfa[5,6], Francesco Bellia[7], Barbara Tomasello[5,6], Daria Nicolosi[2,8], Roberta Malaguarnera[1] , Simone Ronsisvalle[2,9], Fiorella Guadagni[4,10] and Rosaria Acquaviva[2,5,6]

AFFILIATION Academic: [1]Faculty of Medicine and Surgery, "Kore" University of Enna, Italy; [2]Nacture S.r.l., Spin-Off University of Catania, Italy; [3]Department of Biomedical and Biotechnological Sciences, Microbiology Section, University of Catania, Italy; [4]Department of Human Sciences and Quality of Life Promotion, San Raffaele Roma Open University, Rome, Italy; [5]Department of Drug and Health Sciences, Biochemistry Section, University of Catania, Italy; [6]Research Centre on Nutraceuticals and Health Products (CERNUT), University of Catania, Italy; [7]Institute of Crystallography, National Research Council (CNR), Catania, Italy; [8]Department of Drug and Health Sciences, Microbiology Section, University of Catania, Italy; [9]Department of Drug and Health Sciences, Medicinal Chemistry Section, University of Catania, Italy; [10]BioBIM (InterInstitutional Multidisciplinary Biobank), IRCCS San Raffaele Pisana, Rome, Italy

JOURNAL AND BIBLIOGRAPHIC REFERENCES Cells. 2022 May 19;11(10):1696. DOI: 10.3390/cells11101696

STUDY DESIGN Preclinical *in vitro* study

TREATMENT Acetonic extract of *Orobanche crenata* leaves. Total phenols content ranging from 186 ± 1.23 to 209 ± 3.79 µM (as gallic acid)

Main results and author's conclusion

The Authors described the anticancer and antiviral properties of *Orobanche crenata* leaves extract (OCLE, phenols content ranging from 186 ± 1.23 to 209 ± 3.79 µM as gallic acid). After 24 hours of treatment, OCLE did not affect the viability of human fibroblasts HFF-1 at all tested concentrations (from 75 to 1200 µg/ml), but it inhibited succinate dehydrogenase activity of human breast cancer cell line MCF-7 (EC_{50}=396.6 µg/mL). The extract also significantly increased in lactic dehydrogenase release in MCF-7 cells, confirming that its anti-proliferative activity was associated with necrotic cellular death. Furthermore, OCLE treatment resulted in a significant decrease in ROS levels and in a corresponding increase of non-protein thiol levels in MCF-7 cells. The antioxidant activity of the extract was confirmed by the dose-dependent scavenger ability against DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis(ethylbenzothiazoline-6-sulphonic acid). Concerning the antiviral properties, OCLE exhibited better activity against Herpes simplex type 1 (HSV-1), with an ID50 value of 50 µg/mL. For *Herpes simplex* type 2 (HSV-2) and *Coxsackievirus* B1 (COX B1), an ID_{50} value of 100 µg/mL was obtained. A weak activity was observed for *Coxsackievirus* B3 (COX B3) (200 µg/mL). OCLE was inactive against *Poliovirus* 1, *Echovirus* 9, Human Respiratory Syncytial Virus, *Adenovirus* type 2 and type 5 and β-Coronavirus. The properties of the extract could be related to the presence of several bioactive molecules detected by UPLC-MS/MS, including acteoside, luteolin, apigenin and salidroside. In conclusion, these results highlight the potential of OCLE as both a chemotherapeutic and antiviral adjuvant.

Commentary

Natural products represent a therapeutic possibility also against complex pathologies like cancer and viral infections. Moreover, as add-on therapies, they could allow to reduce drug dosage and side effects, strongly improving patient's quality of life. The extracts of the different parts of *O. crenata* showed interesting pharmacological activities, including

antioxidant, anti-inflammatory, antimicrobial and anticancer activities. The Authors showed that OCLE has good antiproliferative activity, being cytotoxic in MCF-7 breast cancer, but not in non cancer cells. Furthermore, the reduction in the intracellular ROS levels and the concomitant increase in thiols availability, proved the ability of the extract to modulate the redox status of breast cancer cells, hindering the carcinogenic effect of oxidative stress. Concerning the antiviral activity, OCLE was found to inhibit the propagation of HSV (1 and 2) and COX (B1 and B3). The antiproliferative and antiviral activities of OCLE can be related to the presence of molecules such as acteoside, apigenin, luteolin and salidroside, whose antioxidant, antiviral and antitumor properties have already been reported. Consequently, it could be interesting in the future to deepen the molecular aspects underlying the antiviral and antiproliferative properties of OCLE and to identify the active ingredient(s) for pharmacological evaluation. For this purpouse, it would be advisable to quantify the molecules contained in OCLE, in order to select the most widely represented.

Mersaquinone, A New Tetracene Derivative from the Marine-Derived *Streptomyces* sp. EG1 Exhibiting Activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

COMMENTED BY Dr. Fortunato Palma Esposito, *Department of ecosustainable marine biotechnology at Stazione Zoologica Anton Dohrn, Naples*

AUTHORS Min Cheol Kim[1], Reiko Cullum[1], Ali M. S. Hebishy[2], Hala A. Mohamed[2], Ahmed H. I. Faraag[3], Nehad M. Salah[1], Mohamed S. Abdelfattah[2], and William Fenical[1]

AFFILIATION Academic: [1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA; [2]Natural Products Research Unit, Chemistry Department, Faculty of Science, Helwan University, Cairo 11795, Egypt; [3]Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo 11795, Egypt

JOURNAL AND BIBLIOGRAPHIC REFERENCES Antibiotics 2020, 9(5), 252; doi.org/10.3390/antibiotics9050252

STUDY DESIGN Preclinical *in vitro* study

TREATMENT Mersaquinone, tetracenomycin D, resistoflavin and resistomycin isolated from the organic extract of the marine *Streptomyces* sp. EG1

Main results and author's conclusion

This paper describes the isolation and structure elucidation of a new tetracene derivative from the culture extract of the marine-derived *Streptomyces* sp. EG1. It reports a classic pipeline for the discovery of novel microbial natural products, starting with the collection of marine sediments, the isolation of microorganisms, the production and extraction of metabolites, followed by purification, identification and evaluation of the biological activity of the pure molecules. Specifically, the producer bacterium was isolated on starch-casein agar plate from sediments collected from the north coast of the Mediterranean Sea of Egypt. The strain was cultivated in a carbon-rich medium (Waksman medium) for 7 days at 28 °C, and the metabolites extracted by XAD-7 resin. The purification of the organic extract led to the isolation of one new compound, i.e. mersaquinone as well as the known tetracenomycin D, resistoflavin and resistomycin, identified by spectroscopic techniques including HRESIMS, IR, 1D and 2D NMR. Mersaquinone was isolated as a red amorphous powder and its molecular formula was assigned as $C_{19}H_{12}O_6$ based on HR-ESI-MS data having the mass $[M+H]^+$ at m/z 337.0714. The new compound showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), with a minimum inhibitory concentration of 3.36 µg/mL (10 µM). This study revealed that sediments of the Mediterranean Sea are an important source of microorganisms, which may produce new compounds with promising biological activities.

Commentary

In this paper, the application of a traditional pipeline for drug discovery successfully led to the identification of a new compound displaying a moderate antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is an infectious multidrug resistant bacterium widely distributed worldwide. The antimicrobial resistance is one of the big-

gest threats to human and animal health today. To counter this phenomenon, the hunt for new antibiotics is spearheaded by natural products discovery. The genus *Streptomyces* is one of the most prolific source of new antibiotics. However, genomic analysis demonstrated that the number of genes responsible for the production of new molecules within this genus far surpasses the number of actual molecules discovered. So, one of the current and future challenges in the field of drug discovery is the induction of the expression of these genes by integrating different approaches and disciplines. The paper also highlights the importance to study the under-exploited Mediterranean Sea as source of new drugs from bacteria.

Gut bacterial metabolite Urolithin A inhibits myocardial fibrosis through activation of Nrf2 pathway in vitro and in vivo

COMMENTED BY Lara Testai, *Department of Pharmacy, via Bonanno, 6-56120 University of Pisa (Pisa) Italy*

AUTHORS Chen P.[1], Pei J.[1], Wang X.[1], Tai S.[1], Tang L.[1], Hu X.[2]

AFFILIATION Academic: [1]Department of Cardiology, The Second Xiangya Hospital of Central South University, No.139, Middle Ren-min Road, Changsha, 410011, Hunan Province, People's Republic of China. [2]Department of Cardiology, The Second Xiangya Hospital of Central South University, No.139, Middle Ren-min Road, Changsha, 410011, Hunan Province, People's Republic of China.

JOURNAL AND BIBLIOGRAPHIC REFERENCES Mol Med. 2022 Feb 8;28(1):19. DOI: 10.1186/s10020-022-00444-1

STUDY DESIGN Preclinical *in vitro/in vivo*

TREATMENT Urolithin A

Main results and author's conclusion

Urolithin A is an active metabolite produced by gut microbiota from gallic acid and ellagitannins, polyphenols present in various fruits, such as pomegranates, strawberries and raspberries, widely consumed in the Mediterranean basin and in the world. Urolithin A is endowed with numerous pharmacological effects, but the molecular targets and mechanisms still remain poorly known. In this study, the Authors demonstrated the involvement of the transcription factor Nrf2 in the modulation of the fibrotic processes triggered by an ischemic event at cardiac level. Specifically, the mechanistic study was carried out on primary cultures of cardiac fibroblasts subjected to TGFbeta1-induced pro-fibrotic stimulation. Urolithin A has been shown to inhibit concentration- and time-dependently cell proliferation, migration and invasion. The analysis of fibrosis markers (including alpha-SMA and vimentin) showed an attenuation in their expression; moreover, an increased expression of Nrf2 and of the downstream markers, including SOD1, HO-1 and NQO1, was observed. The involvement of Nrf2 was confirmed by its silencing in cardiac fibroblasts. The critical role of Nrf2 in the anti-fibrotic effects of urolithin A was also confirmed in a murine model of myocardial infarction *in vivo*.

Commentary

The identification of the molecular mechanisms underlying the pharmacological effects of extracts and/or substances of natural origin represents a fundamental step in order to understand their real translational value and to use them successfully in the prevention/treatment of pathological conditions. In this regard, myocardial infarction represents one of the main cardiovascular diseases associated with reduced life expectancy. A consequence of myocardial infarction, in surviving patients, is the fibrosis which reduces cardiac performance and leads to heart failure over the time. Of note, *in vitro* and *in vivo* treatment with urolithin A showed to control cardiac fibrosis processes, with Nrf2 emerging as a central actor. Therefore, this study contributes to the knowledge of the clinical benefits obtainable through the consumption of plants naturally rich in gallic acid and ellagitannins.

Anticandidal activity of dichloromethane extract obtained from the red algae *A. armata* of the Algerian coast

COMMENTED BY Marco Micera, *PhD and researcher at Exenia Group s.r.l. Pinerolo (TO), Italy*

AUTHORS Ilhem Messahli[a], Hicham Gouzi[a], Ibrahim Sifi[b], Rachid Chaibi[a], Asma Rezzoug[a], Linda Rouari[a]

AFFILIATION Academic: [a]Laboratoire des Sciences Biologiques et Agronomiques (LSBA), Faculté des Sciences, Université Amar Télidji, Route de Ghardaïa BP37G (03000), Laghouat, Algeria. [b]Laboratoire des Sciences Fondamentales à l'Université Amar Télidji, Route de Ghardaïa BP37G (03000), Laghouat, Algeria.

JOURNAL AND BIBLIOGRAPHIC REFERENCES Acta Ecologica Sinica, 2021, doi.org/10.1016/j.chnaes.2021.08.005

STUDY DESIGN Preclinical *in vitro* study

TREATMENT Dichloromethane algal extract

Main results and author's conclusion

The Authors evaluated, at preliminary level, the antifungal activity of an algal extract obtained by using dichloromethane (DCM) as a solvent. The algal species was the Algerian invasive red seaweed *Asparagopsis armata*, while the cellular model used consisted in two strains of *Candida albicans*, namely (IP 444) and (ATCC 10231), mainly known for drug resistance phenomenon. According to numerous other publications, antifungal activity was expressed as zone of growth inhibition through diffusion tests in Petri dishes containing Sabouraud Dextrose Agar (SDA) medium. The minimum inhibitory concentration (MIC) for the two fungal strains was also evaluated. In this context, the DCM extract of the red alga *A. armat*a had a strong inhibitory effect against the two *C. albicans* strains examined. Furthermore, *C. albicans* IP444 (MIC = 0.58 mg/mL) was more sensitive to DCM algae extract than *C. albicans* ATCC 10231 (MIC = 2.34 mg/mL). GC-MS analysis suggested that brominated compounds and fatty acids were responsible for the antifungal activity of the extract. Overall, these preliminary results suggest that DCM extract from the red algae *A. armata* has promising antifungal activities, useful for counteracting the increasingly worrying phenomenon of drug resistance.

Commentary

The valorization of plant biomass is becoming increasingly important nowadays, even more if they are harmful to the environment and if they must be disposed of through special and expensive procedures. In this article, the Authors evaluated the potential biological activities of an algal extract obtained from *Asparagopsis armata* biomass, an invasive Algerian red seaweed that must be periodically removed from many kilometers of the Mediterranean coast. By using this biomass as an extractive matrix, it is possible to obtain extracts with important biological properties, and add value to a biomass that is especially problematic at an environmental level. The antifungal results obtained, using dichloromethane as an extractive solvent, were clearly demonstrated on (IP 444) and (ATCC 10231) *Candida albicans* strains by diffusive methods with agar. Probably, the antifungal activity of the extract may be attributable to the presence of lipophilic halogenated compounds such as bromoform, which expresses an important antimethanogenic activity responsible for the anticandidal activity. To better understand this activity and to continue with clinical studies, it is necessary to proceed with isolation, purification and identification of the bioactive compounds and, subsequently, with toxicological analyzes.