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Potential anticancer activity of lichen secondary metabolite physodic acid

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ABSTRACT

Secondary metabolites present in lichens, which comprise aliphatic, cycloaliphatic, aromatic and terpenic compounds, are unique with respect to those of higher plants and show interesting biological and pharmacological activities. However, only a few of these compounds, have been assessed for their effectiveness against various *in vitro* cancer models. In the present study, we investigated the cytotoxicity of three lichen secondary metabolites (atranorin, gyrophoric acid and physodic acid) on A375 melanoma cancer cell line. The tested compounds arise from different lichen species collected in different areas of Continental and Antarctic Chile. The obtained results confirm the major efficiency of depsidones. In fact, depsides atranorin and gyrophoric acid, showed a lower activity inhibiting the melanoma cancer cells only at more high concentrations. Whereas the depsidone physodic acid, showed a dose-response relationship in the range of 6.25–50 μM concentrations in A375 cells, activating an apoptotic process, that probably involves the reduction of Hsp70 expression. Although the molecular mechanism, by which apoptosis is induced by physodic acid remains unclear, and of course further studies are needed, the results here reported confirm the promising biological properties of depsidone compounds, and may offer a further impulse to the development of analogues with more powerful efficiency against melanoma cells.

1. Introduction

Lichens are complex symbiotic organisms of fungi and algae. They are the earliest colonizers of terrestrial habitats on earth, and they show a worldwide distribution from arctic to tropical regions. In particular they are the most conspicuous macroscopic organisms in Continental South America (Chile) and in Antarctica, in terms of species, biomass and distribution [1]. Lichens and their metabolites have long been used by humans. Throughout the ages lichen extracts have been used for various purposes, in particular as dyes, perfumes and for various remedies in folk medicine since ancient Egyptian times [2]. Chemical studies on the secondary metabolites present in lichens have led to the isolation of many new substances, which by today number over 800 [1,2]. Many of these compounds, including depsides, depsidones, usnic acids, dibenzofuranes, xanthones, anthraquinones, pulvinic acid derivatives and aliphatic acids, are unique with respect to those of higher plants and show interesting pharmacological activities [1,2]. In fact, in addition to their natural role, lichen secondary compounds have shown a variety of biological activities such as, antibiotic, antimycobacterial, antimutagenic, antioxidant, antiviral, antipyretic, analgesic and antitumoral properties, and have been used for treatment of various conditions in traditional medicine [3]. Although many natural and cultured lichens have been studied for their biological properties and several compounds have been purified and identified, their therapeutic potential has not yet been fully explored. Particularly, limited informations are available on the anticancer effects of pure compounds obtained from lichens. Usnic acid, the prototype of low-molecular weight compounds derived from lichens, has been the most extensively studied. It is used in pharmaceutical preparations against infections, bacterial eczema, mastitis, furunculosis and polydermy [4]. In addition, preclinical studies have permitted to hypothesize its possible use as a antineoplastic agent [5]. The toxicity of usnic acid was associated with increased P450 activity and oxidative stress in human hepatoblastoma cells [6], with mitochondrial dysfunction in HepG2 cells

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[6], in the breast cancer T-47D cell line and in the pancreatic cancer Capan-2 cell line [7], with apoptotic induction in murine leukaemia L1210 cells [8]. In addition to this dibenzofuran derivative, also several well-characterized depsides and depsidones exhibit anti-cancer properties [1]. Sphaerophorin and pannarin inhibited the growth of human prostate carcinoma DU-145 and melanoma M14 cells, inducing apoptotic cell death [9,10]. Also vicanicin, protolichesterinic and lobaric acids showed significant anti-proliferative effects against a variety of human cancer cell lines [11–14]. Lichen derived compounds, are promising agents in the inhibition of cell proliferation. We therefore decided to extend the study on lichen compounds investigating the effect of three secondary metabolites the depsides atranorin (**1**) and gyrophoric acid (**2**) and the depsidone physodic acid (**3**) (Fig. 1), obtained from different lichen species collected in many places of Continental and Antarctic Chile, on cell growth and death in A375 melanoma cancer cell line.

2. Materials and methods

2.1. Chemicals

All reagents were of commercial quality and were used as received. 3 (4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) and β-nicotinamide-adenine dinucleotide (NADH) were obtained from Sigma Aldrich Co (St. Louis, USA). All other chemicals were purchased from Sigma Aldrich Co (St. Louis, USA) and GIBCO BRL Life Technologies (Grand Island, NY, USA).

2.2. Plant material

The tested compounds arise from diverse lichen species collected in different localities of Continental and Antarctic Chile. Atranorin (**1**) was isolated from *Bacidia stipata* as previously described [15]. Gyrophoric acid (**2**) was isolated from *Ochrolechia deceptionis* Hue. collected on King George Island, Antarctic and *Placopsis contortuplicata* Lamb. from Robert Island, Antarctic. Physodic acid (**3**) was isolated from *Hypogymnia lugubris* (Pers.) Krog collected on King George Island, South Shetland Islands, Antarctic. General experimental details have been reported previously [16,17]. The compounds, after extraction from all lichens, were isolated by chromatography using Si gel column, and identified by spectroscopic techniques, by comparison with authentic samples. Their purity grade was 99%. IR Infrared spectra were acquired by Perkin Elmer Spectrum One using KBr pellet method. The matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were acquired by a Voyager DE-STR (PerSeptive Biosystem) using a simultaneous delay extraction procedure (20 kV applied after 233 ns with a potential gradient of 2545 V/mm and a wire voltage of 200 V) and detection in reflection mode. The instrument was equipped with a nitrogen laser (emission at 337 nm for 3 ns) and a flash AD converter (time base 2 ns). *Trans*-3-Indoleacrylic acid (IAA) was used as a matrix; the average molecular masses were determined using a Grams/386 program (by PerSeptive Biosystem).

2.3. Study on human tumor cell line

2.3.1. Cell culture and treatments

A375 human melanoma cell line was obtained from American Type Culture Collection (Rockville, MD, USA). A375 cell line was grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 2.0 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 25 μg/mL fungizone (Sigma-Aldrich, Italy). Normal human non-immortalised buccal fibroblast cells, kindly donated by Institute IGB, CNR (Naples, Italy), were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml

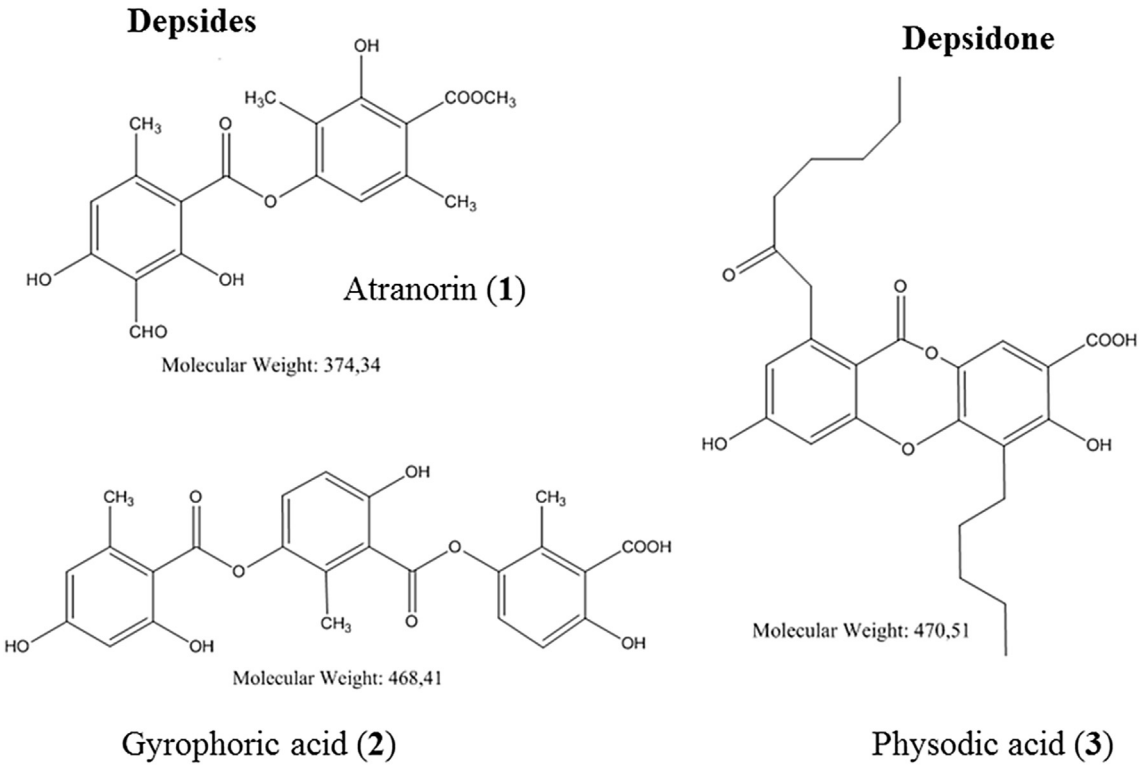

Fig. 1. The structure of the depsides atranorin (**1**) and gyrophoric acid (**2**) and the depsidone physodic acid (**3**).