Pharmacology

Chemotherapy

Chemotherapy 2009;55:60–68
DOI: 10.1159/000180340

Received: May 22, 2008
Accepted after revision: October 15, 2008
Published online: December 8, 2008

Antiproliferative, Cytotoxic and Hemolytic Activities of a Triterpene Glycoside from *Psolus patagonicus* and Its Desulfated Analog

Valeria P. Careaga[a] Carlos Bueno[b] Claudia Muniain[c] Laura Alché[b]
Marta S. Maier[a]

[a]UMYMFOR (CONICET-UBA) y Departamento de Química Orgánica, y [b]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, [c]Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Campus Miguelete, Buenos Aires, Argentina

Key Words
Triterpene glycosides · Sea cucumber · *Psolus patagonicus* · Nuclear factor-kappa B · Antiproliferation · Cytotoxicity

Abstract
Background: The major triterpene glycoside of the sea cucumber *Psolus patagonicus* and its desulfated analog were tested for their antiproliferative, cytotoxic and hemolytic activities, and their effect on NF-κB activation. ***Methods:*** The antiproliferative action of glycosides 1 and 2 were determined on 3 tumor cell lines. Their effect on the activation of NF-κB was evaluated by indirect immunofluorescence assay staining and the concomitant IκBα degradation was studied by Western blot. ***Results:*** Both compounds were able to suppress the growth of 3 tumor cell lines (Hep3B, MDA-MB231 and A549) and induced the activation of NF-κB, a key player linking chronic inflammation and cancer, concomitant with IκBα degradation in the A549 tumor cell line. Compounds 1 and 2 showed hemolytic activity with half maximal inhibitory concentration (IC_{50}) values around 80 μM. ***Conclusions:*** Both glycosides showed low cytotoxic activity in A549 tumor cells in comparison with sea cucumber triterpene glycosides containing a linear tetrasaccharide chain. This could be a result of the uncommon presence of two 12α- and 17α-hydroxyl groups and a Δ^7 double bond in the aglycone moiety. This aglycone functionalization may be related to their low membranolytic activity. Although glycosides 1 and 2 exert an antiproliferative effect, their mechanisms of action do not involve inhibition of NF-κB. Recently, it has been shown that diverse and new mechanisms of action are responsible for the antitumor and cytotoxic activities of marine compounds. Therefore, more extensive studies are needed to establish a mechanism of action and to deduce a clear structure-activity relationship of sea cucumber triterpene glycosides.

Introduction

Nature has supplied several anticancer agents and continues to be an exceptional reservoir of bioactive compounds [1, 2]. Marine organisms are sources of new natural products with unique structural features, many of which exhibit potent pharmacological activities [3, 4]. Echinoderms belonging to the classes Asteroidea (starfishes or sea stars) and Holothuroidea (sea cucumbers) produce complex mixtures of saponins that are responsible for their general toxicity [5, 6]. These secondary metabolites are very common in plants, but their presence in the animal kingdom is very rare.

Holothurins isolated from sea cucumbers are triterpenoid oligoglycosides that contain an aglycone based on a 'holostanol' skeleton (3β,20*S*-dihydroxy-5α-lanostano-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0009–3157/09/0551–0060$26.00/0

Accessible online at:
www.karger.com/che

Prof. Dr. Marta S. Maier
UMYMFOR (CONICET-UBA), Departamento de Química Orgánica
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires (Argentina)
Tel./Fax +54 11 4576 3385, E-Mail maier@qo.fcen.uba.ar

18,20-lactone) and a sugar chain of 2–6 monosaccharide units linked to the C-3 of the aglycone. Quinovose, glucose, 3-*O*-methylglucose, xylose and rarely 3-*O*-methylxylose are present in the carbohydrate moieties of these glycosides. Triterpene glycosides are produced in the skin and in the Cuvier's tubules of sea cucumbers and are ejected when the animals are disturbed. This behavior may be associated with a defensive function due to the ability of holothurins to form complexes with cholesterol and other Δ^5 sterols from cell membranes. This membranolytic activity probably determines the wide spectrum of their biological effects, including their antifungal, antiviral, ichthyotoxic, hemolytic and immunomodulatory properties [7]. Several sea cucumber triterpene glycosides have shown cytotoxic activities on early embryogenesis and against human and mice tumor cell lines [7–10].

As a continuation of our search for bioactive metabolites from the cold water echinoderms of the South Atlantic [11–14] we have isolated Patagonicoside A (1), the main holothurin from the Patagonian sea cucumber *Psolus patagonicus* (fig. 1) [15]. Compound 1 is a disulfated triterpene tetraglycoside with an uncommon aglycone with 2 hydroxyl groups at C-12α and C-17α, a characteristic of aspidochirotid sea cucumbers, and a Δ^7 double bond. Glycoside 1 and its desulfated semisynthetic analog 2 have shown antifungal activity against the phytopathogenic fungi *Cladosporium cucumerinum* [15], *Cladosporium fulvum*, *Fusarium oxysporum* and *Monilia* species [16]. The aim of the present study was to investigate the effect of triterpene glycoside 1 and its desulfated analog 2 on the proliferation and viability of different human tumor cell lines, on the activation of nuclear factor-kappa B (NF-κB), and their hemolytic activity on erythrocytes from mouse blood.

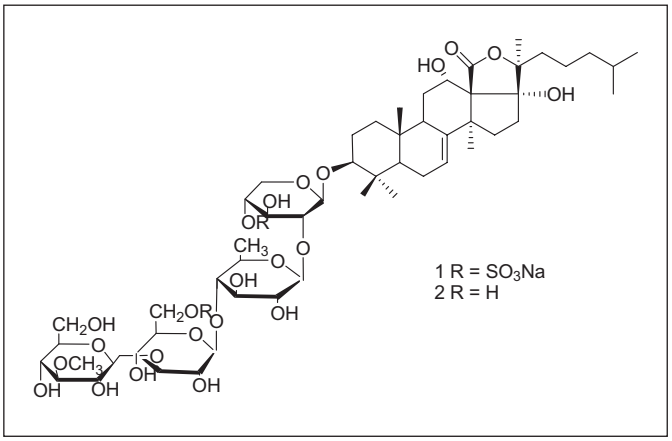


Fig. 1. Structure of the main component, Patagonicoside A (1), and its desulfated derivative (2).

Materials and Methods

Triterpene Glycosides

In this study the triterpene glycosides Patagonicoside A and its desulfated analog were tested. For details on the extraction and purification of Patagonicoside A from the sea cucumber *P. patagonicus* and its desulfation reaction, see Murray et al. [15]. Chemical structures and purities of the isolated compounds were confirmed by RP-HPLC and ^{1}H- and ^{13}C-NMR.

Cell Culture and Reagents

Human hepatocellular carcinoma Hep3B cells were grown in Eagle's minimal essential medium (MEM) supplemented with 10% inactivated fetal bovine serum (MEM 10%; Natocor, Córdoba, Argentina), sodium pyruvate (1 mM) and L-glutamine (2 mM; Gibco, Invitrogen Corp., Rockville, Md., USA). Human mammary gland adenocarcinoma MDA-MB231 cells were grown in RPMI 1640 medium supplemented with 10% inactivated fetal bovine serum (RPMI 10%; Gibco, Invitrogen Corp.). Human lung carcinoma A549 cells were grown in MEM 10%. Cell cultures were maintained in a 4% CO_2 humidified atmosphere at 37°C. Tumor necrosis factor-α (TNF-α) mouse recombinant expressed in *Escherichia coli* was purchased from Sigma (Saint Louis, Mo., USA).

Antiproliferative Assay

We seeded 2.4×10^6 cells in 96-well plates (Costar, Corning Inc., Corning, N.Y., USA) together with different concentrations of triterpene glycosides 1 and 2 in duplicate, and incubated at 37°C for 24 h in a 4% CO_2 atmosphere. Then, cells were fixed with 10% formaldehyde for 15 min at room temperature, washed once with distilled water and stained with 0.05% crystal violet in 10% ethanol over 30 min. Afterwards cells were washed once and eluted with a solution of 50% ethanol and 0.1% acetic acid in water. The absorbance of each well was measured on an Eurogenetics MPR-A 4i microplate reader using a test wavelength of 590 nm. Results were expressed as the percentage of absorbance of treated cells with respect to untreated ones, and we considered the untreated control cells as 100% of cell survival. The half maximal inhibitory concentration (IC_{50}) was defined as the concentration of compound that caused a 50% reduction in cell survival.

Cytotoxicity Assay

Cell viability was determined as previously reported [17]. Briefly, cell viability in the presence of the compound was determined using the cleavage of the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma] by the mitochondrial enzyme succinate dehydrogenase to give a blue product (formazan). The absorbance of each well was measured on an Eurogenetics MPR-A 4i microplate reader using a test wavelength of 570 nm and a reference wavelength of 630 nm, by duplicate. Results were expressed as a percentage of absorbance of treated cells with respect to untreated ones and we considered the untreated control cells as 100% of cell survival. The CC_{50} was de-

fined as the concentration of compound that caused a 50% reduction in cell viability.

Indirect Immunofluorescence Assay

Subconfluent A549 cells grown on glass coverslips in 24-well plates (Costar, Corning Inc.) over 24 h were fixed with methanol for 10 min at –20°C. After 3 washes with phosphate-buffered saline (PBS), the coverslips were inverted on a drop of 1/100 diluted primary rabbit polyclonal anti-p65 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif., USA) for 30 min at 37°C, and then returned to culture dishes and subjected to 3 additional washes with PBS. Afterwards, cells were incubated with 1/50 diluted secondary goat anti-rabbit FluoroLink™ Cy™2 (GE Healthcare Bio-Sciences, Buenos Aires, Argentina), for 30 min at 37°C. Finally, coverslips were rinsed, mounted and photographed with a Zeiss microscope with epifluorescence optics. Images were collected with a 40× objective from the tissue locations and imported into the NIH ImageJ 1.34s program (written by Wayne Rasband, National Institute of Mental Health, Bethesda, Md., USA). The immunofluorescence images were converted to 8-bit grayscale, from 0 (black) to 255 (white), and magnified. Individual cells were mapped for total or nuclear fluorescence and the mean density from each one was obtained. This process was repeated for several cells in the field, and the average was calculated to represent the total and/or nuclear mean density for an individual cell. To compare fluorescence distribution within the cell, numerical results were put in a table in Excel. Total intensity was calculated as the total mean density per total area, and nuclear intensity was calculated as the nuclear mean density per nuclear area. Then, the percentage of intensity in the nucleus with respect to total intensity was estimated.

Western Blot Analysis

Whole extracts from cells grown in 24-well plates for 24 h were loaded on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes for 60 min at 75 mA. Membranes were blocked in PBS containing 5% unfiltered milk overnight and then incubated with 1/500 diluted anti-IkBα antibodies (Santa Cruz Biotechnology), 1/3,000 diluted anti-calnexin antibodies (Chemicon, Temecula, Calif., USA) or 1/2,000 diluted anti-actin antibodies (kindly provided by Viviana Castilla, Laboratory of Virology, School of Natural and Exact Sciences, University of Buenos Aires, Argentina). After washing, membranes were incubated with 1/10,000 diluted peroxidase-conjugated goat anti-rabbit or anti-mouse (ICN Immunobiological, Tel Aviv, Israel). The immunoreactive bands were visualized using an enhanced chemiluminescence system (ECl, PerkinElmer, Boston, Mass., USA).

Hemolytic Activity

The hemolytic activity was determined as previously reported [18]. Erythrocytes were isolated from mouse blood, washed 3 times with PBS (pH 7.4) using centrifugation (450 *g*, 10 min), and the residue of erythrocytes was resuspended in PBS to a final concentration of 1.0% and kept on ice.

For the hemolytic assay, 100 μl of aqueous solution of the tested substances 1 and 2 having different concentrations was mixed with 900 μl of erythrocyte suspension and incubated at 37°C for 1 h. The residual cells were sedimented by centrifugation, aliquots of supernatant (200 μl) were transferred to the wells of 96-well microplates and the hemoglobin concentration in the supernatant was evaluated spectroscopically at λ_{ex} = 550 nm. The results were expressed as percent of hemolysis.

Results

Antiproliferative Effect and Cytotoxicity of Triterpene Glycosides 1 and 2 on Tumoral Cells

Three tumorigenic cell lines (Hep3B, MDA-MB231 and A549) were chosen to determine the antiproliferative activity of triterpene glycosides 1 and 2. After a 24-hour incubation, the percentage of cells surviving decreased gradually with the increase in the concentration of triterpene glycosides 1 and 2. The IC_{50} values of compound 1 in Hep3B, MDA-MB231 and A549 cell lines were similar (15.89, 19.62 and 15.04 μM, respectively; fig. 2). The desulfated analog 2 exhibited a slightly stronger antiproliferative effect for Hep3B (fig. 2b) and MDA-MB231 (fig. 2c) cells, since their IC_{50} values were 5.09 and 5.39 μM, respectively, although a concentration of 16.53 μM was enough to provoke a 50% reduction in A549 cell survival (fig. 2a). These results demonstrate that triterpene glycosides 1 and 2 are able to suppress the growth of different tumor cell lines. Taking into account that both glycosides showed similar IC_{50} values in A549 cells, we chose this cell line to study the biological action of compounds 1 and 2 at intracellular levels. For that reason, we determined first the cytotoxic activity of both compounds on this cell line, which dose-dependently inhibited cell viability (fig. 3). We found that CC_{50} values were 44 and 14.6 μM for triterpene glycosides 1 and 2, respectively.

Effect of Triterpene Glycosides 1 and 2 on NF-κB Activation

Constitutive NF-κB activation is likely involved in the enhanced growth properties seen in a variety of cancers [19]. The ability of NF-κB to inhibit apoptosis, as well as to promote cell proliferation, makes it an attractive target for cancer therapy [20, 21]. To investigate the effect of triterpene glycosides 1 and 2 on the activation of NF-κB, the corresponding IC_{50} values (15.04 and 16.53 μM) were added to A549 cells, and then incubated at 37°C for 30 min or 2 h. Afterwards we performed an indirect immunofluorescence assay staining, as described in Materials and Methods. By visual inspection of the images, we observed that the p65 subunit of NF-κB appeared in the nuclei of treated cells (fig. 4). To corroborate these qualitative observations, a semiquantitative analysis of nuclear

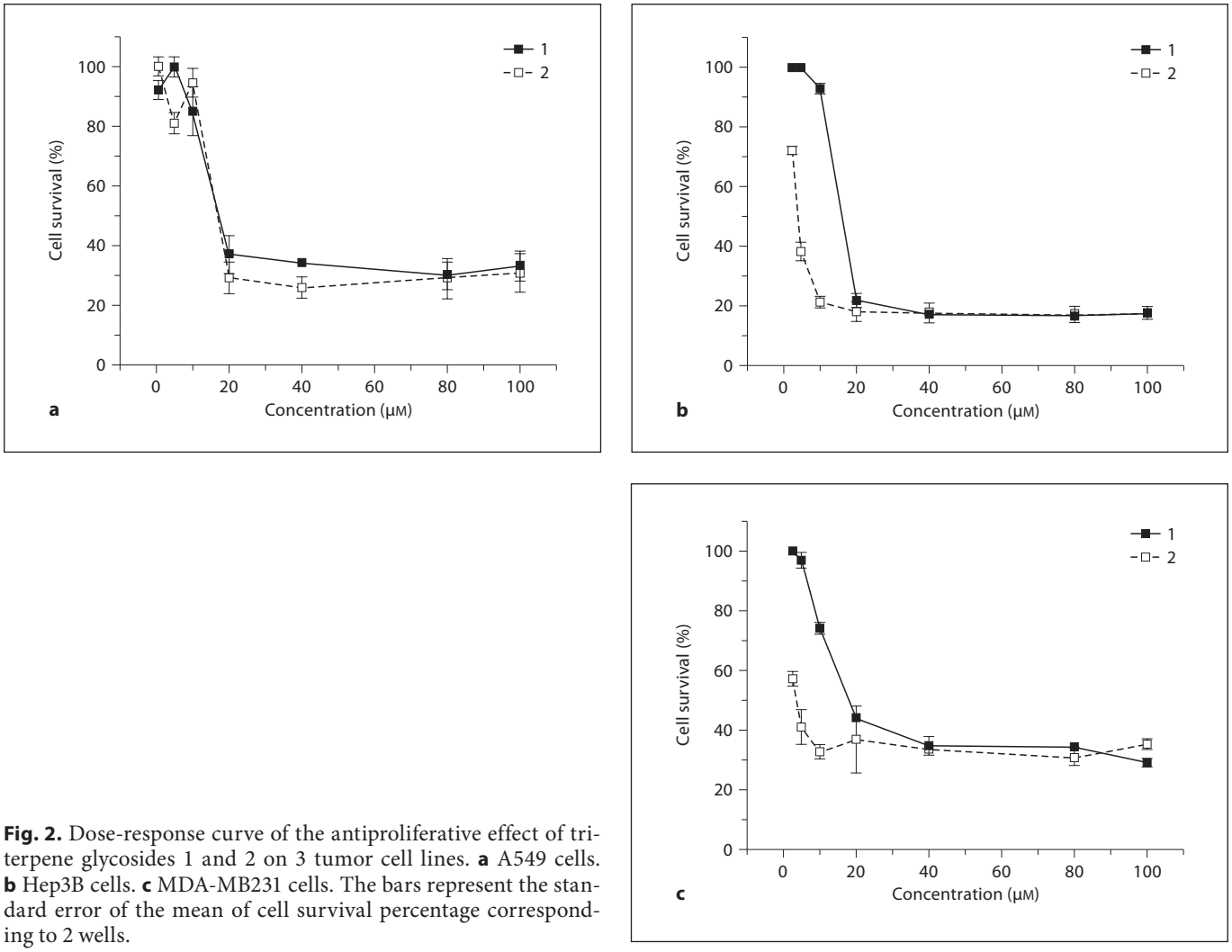


Fig. 2. Dose-response curve of the antiproliferative effect of triterpene glycosides 1 and 2 on 3 tumor cell lines. **a** A549 cells. **b** Hep3B cells. **c** MDA-MB231 cells. The bars represent the standard error of the mean of cell survival percentage corresponding to 2 wells.

translocation of p65 was obtained by counting 20 cells per coverslip, in duplicate, and calculating the percentage of translocation as the number of cells with fluorescence in the nucleus relative to the total cell number.

Both triterpene glycosides induced the activation of NF-κB, since the p65 subunit was detected in the nucleus of 100% of compound 2-treated cells, regardless the time of treatment, whereas NF-κB translocated in 100 and 78% of cells treated with compound 1 for 30 min and 2 h, respectively (fig. 4).

It is well known that NF-κB activity can be induced in most cell types upon treatment with different stimuli, such as TNF-α. Therefore, we decided to provoke NF-κB translocation in A549 cells using this cytokine, which was added to cell cultures for incubation at 37°C. We determined that the induction with 10 ng/ml of TNF-α during 30 min elicited NF-κB translocation in 100% of cells (fig. 5). Similar results were obtained when cells were incubated simultaneously with TNF-α and Patagonicoside A or its desulfated analog. Pre-incubation either with compound 1 or 2 for 2 h, followed by stimulation with TNF-α for 30 min, provoked NF-κB translocation in 92 and 89% of cells, respectively (fig. 5). These results indicate that both compounds activate the NF-κB pathway and none of them inhibit the activation of NF-κB induced by TNF-α. The fact that triterpene glycoside 2 exhibits similar CC_{50} (14.6 μM) and IC_{50} (16.53 μM) values when assayed on A549 cells would account for the differences in the number and cellular morphology corresponding to compound 2-treated cells observed in indirect immunofluorescence assays (fig. 4, 5).

Activity of Triterpene Glycosides 1 and 2 on IκBα Degradation

Since the translocation of NF-κB to the nucleus is preceded by the proteolytic degradation of its inhibitor in the cytoplasm, IκBα, we determined whether NF-κB activation by compounds 1 and 2 was concomitant to IκBα degradation. First, we treated A549 cells with compounds 1 and 2 (15.04 and 16.53 μM, respectively), together with 10 ng/ml TNF-α or not, for 30 min, and examined IκBα status in whole cell extracts, by Western blot analysis. TNF-α induced IκBα degradation in control cells after 30 min of stimulation, in accordance with NF-κB translocation observed through indirect immunofluorescence assay staining (fig. 5, 6). As expected, triterpene glycosides 1 and 2 provoked IκBα degradation as TNF-α did, either alone or in combination with 10 ng/ml TNF-α after 30 min of treatment (fig. 6). When cells were pretreated during 2 h with either or both compounds, with or without the subsequent addition of TNF-α, similar results were obtained (fig. 7).

Hemolytic Activity of Glycosides 1 and 2

The hemolytic activities of a series of triterpene glycosides from sea cucumbers belonging to the order Dendrochirotida have been reported [18]. Thus, we studied the eventual hemolytic activities of compounds 1 and 2. The hemolytic activity started to be detected in the concentration range of 1–3 μM and the IC_{50} values corresponding to the hemolytic activity of compounds 1 and 2 were estimated as 82 and 87 μM, respectively.

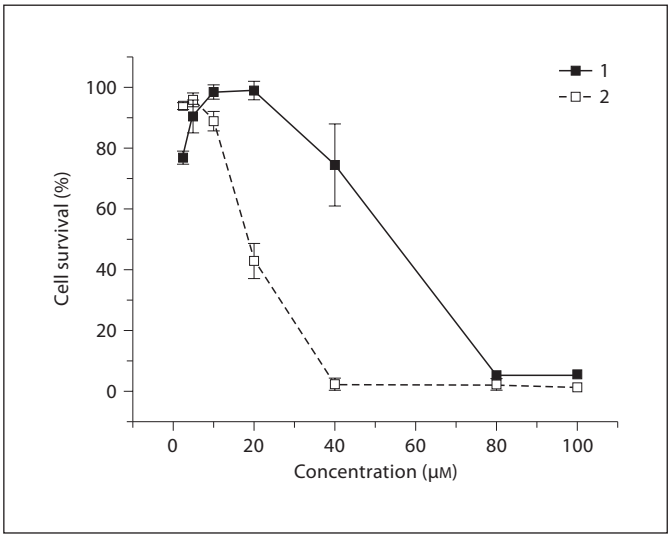


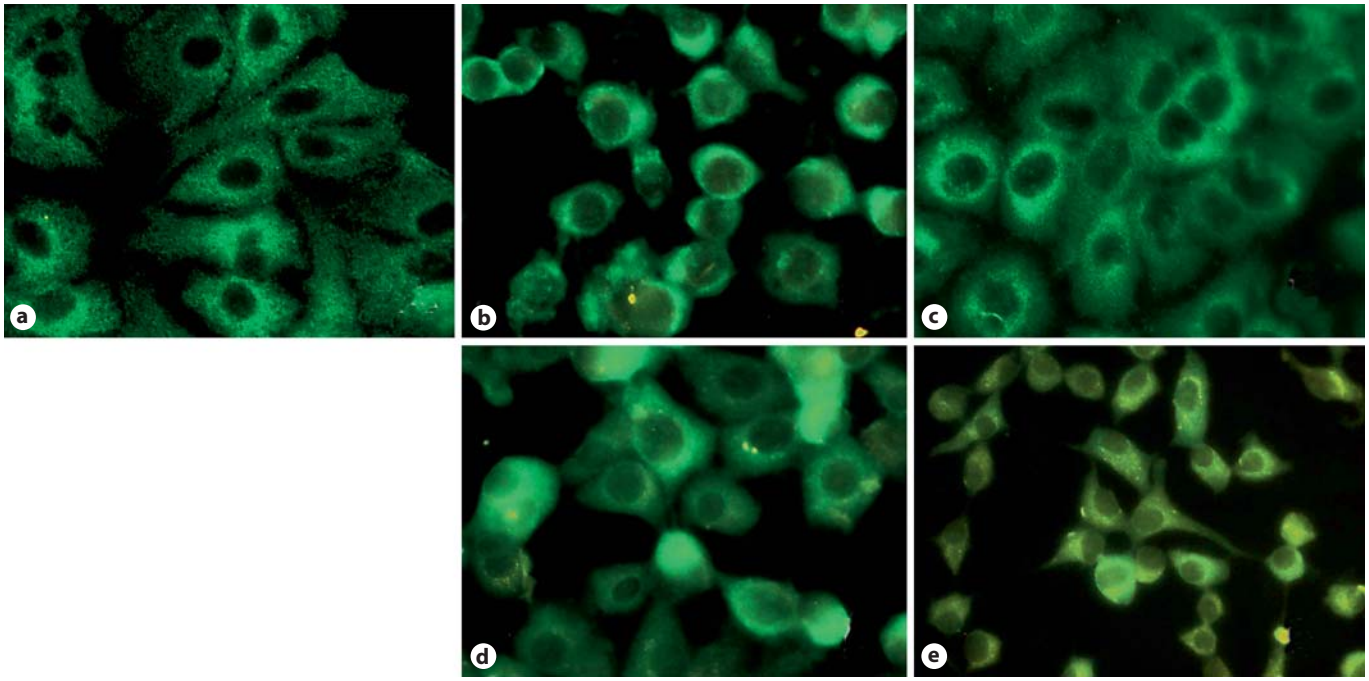
Fig. 3. Dose-response curve of the cytotoxic effect of triterpene glycosides 1 and 2 on A549 cells. The bars represent the standard error of the mean of cell survival percentage corresponding to 2 wells.

Discussion

In the present study it was demonstrated for the first time that Patagonicoside A, a disulfated triterpene tetraglycoside isolated from the sea cucumber *P. patagonicus*, and its desulfated analog showed antiproliferative activity in 3 tumor cell lines (Hep3B, MDA-MB231 and A549). Usually, the literature deals mainly with sea cucumber triterpene glycosides exhibiting cytotoxic activity in tumor cells rather than antiproliferative effects. Recently, Roginsky et al. [22] showed that frondoside A, a monosulfated triterpene pentaglycoside isolated from the sea cucumber *Cucumaria frondosa*, inhibited pancreatic cancer cell growth and induced apoptosis in vitro and in vivo, while Silchenko et al. [23] reported the effects of this pentaglycoside on AP-1-, NF-κB- and p53-dependent transcriptional activities.

Several triterpene glycosides containing a linear tetrasaccharide chain have shown cytotoxic activity on tumor cell growth in vitro. In particular, intercedensides A, B, C, D, E, F, G and H isolated from *Mensamaria intercedens* [24, 25] and Phylinopsides A and B from *Pentacta quadrangularis* [26] showed significant cytotoxicity against A549 tumor cells with effective doses (ED_{50}) in the range 0.7–1.8 μg/ml. The fact that compounds 1 and 2 displayed lower levels of cytotoxicity (IC_{50} in the range 15–16 μM) than most triterpene glycosides already reported [7], together with their remarkable antiproliferative effect in 3 tumor cell lines, suggests that they are more suitable molecules than other sea cucumber triterpene glycosides to impede tumor cell growth in vitro. Besides, differences in the cytotoxicities of the compounds on A549 cells may be related to the presence of sulfate groups in the carbohydrate chain (fig. 3). Similar results were obtained for desulfated Cucumechinosides A, B and C which showed higher cytotoxicity against L1210 and KB cells than the native glycosides [27].

Sea cucumber triterpene glycosides have strong membranotropic effects against cellular membranes containing Δ^5 and $\Delta^{5,7}$ sterols. The formation of complexes with these sterols modifies the structural organization of cellular membranes with the formation of single ion channels and larger pores [7]. These effects probably determine their biological role and correlate with their cyto-



Color version available online

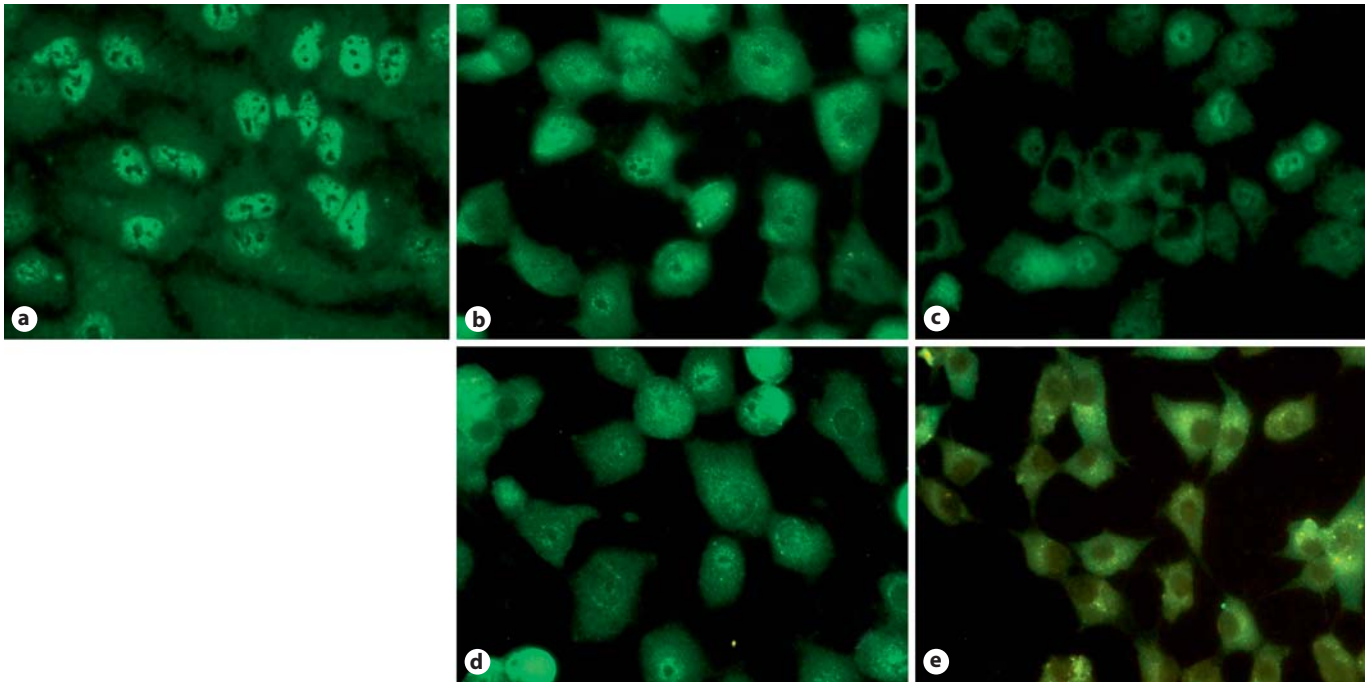
Fig. 4. Effect of triterpene glycosides 1 and 2 on NF-κB nuclear translocation in A549 cells. A549 cells were treated either with compound 1 or 2 during different times. The localization of p65 was detected by IFI staining in methanol-fixed cells. Magnification: 400×. **a** Untreated control cells. **b**, **c** Cells treated with compound 1 for 30 min and 2 h, respectively. **d**, **e** Cells treated with compound 2 for 30 min and 2 h, respectively. Two indirect immunofluorescence assays were performed.

toxicity in tumor cells. Sea cucumbers are resistant to their own toxins due to a low content of free Δ^7 sterols and sulfated and glycosylated sterols in their membrane lipids instead of Δ^5 sterols. Studies on the structure-activity relationship for sea cucumber glycosides revealed that their biological activities depend on both the aglycone and the carbohydrate structures. An 18(20)-lactone in the aglycone moiety is an important requirement for membranotropic action, together with the presence of a linear tetrasaccharide fragment in the carbohydrate chain. Besides, glycosides containing quinovose as the second monosaccharide unit are the most active [7]. Patagonicoside A (1) and its desulfated analog (2) contain these favorable structural features. Nevertheless, both glycosides showed low hemolytic activity in comparison with sea cucumber triterpene glycosides containing a linear tetrasaccharide chain [7].

This could be assessed to the uncommon presence of two 12α- and 17α-hydroxyl groups and a Δ^7 double bond in the aglycone moiety and may be related to their lower level of cytotoxicity.

Recently, Zhang et al. [28] reported a large difference in cytotoxicity against HL-60 cells of two triterpene glycosides which differed only in the presence of a hydroxyl group at C-17 in one of the compounds. However, both glycosides showed similar cytotoxic effects in another cell line, BEL-7402. Liu et al. [29] also reported a lower cytotoxicity of Arguside A against HL-60 cells in comparison with HCT-116, BEL-7042 and MKN-45 cell lines. These facts suggest that different tumor cell lines exhibit a differential sensitivity to the cytotoxic effects of sea cucumber glycosides, which can be related to their chemical structures. The differences in the antiproliferative activities of glycoside 2 observed may be ascribed to a higher sensitivity of A549 tumor cells when treated with compound 2 (fig. 2).

It is well known that cellular proliferation involved in carcinogenesis may be regulated by NF-κB [30]. It has been found that cytokines and cytokine receptors either are regulated by NF-κB or mediate proliferation through activation of NF-κB [31]. On the other hand, NF-κB is important for survival and proliferation of transformed cells and, later on, it is associated with the tumor's ability to invade surrounding tissues and metastasize [19]. For that reason, NF-κB has become a focal point for antitumoral drug discovery, and many compounds of natural



Color version available online

Fig. 5. Effect of triterpene glycosides 1 and 2 on TNF-α-induced NF-κB nuclear translocation in A549 cells. A549 cells were stimulated with 10 ng/ml TNF-α during 30 min, and treated simultaneously or pretreated for 2 h either with compound 1 or 2. The localization of p65 was detected by indirect immunofluorescence assay staining in methanol-fixed cells. Magnification: 400×. **a** TNF-α-treated cells. **b** TNF-α + compound 1. **c** Compound 1 (2 h) + TNF-α. **d** TNF-α + compound 2. **e** Compound 2 (2 h) + TNF-α. Two indirect immunofluorescence assays were performed.

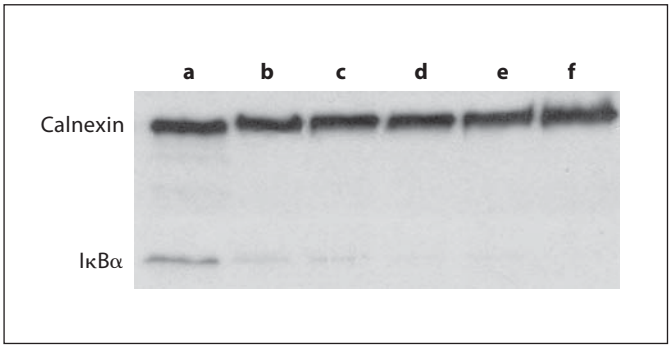


Fig. 6. Effect of triterpene glycosides 1 and 2 on TNF-α-induced IκBα degradation in A549 cells. A549 cells were treated either with compound 1 or 2 together with 10 ng/ml TNF-α or not during 30 min. The detection of IκBα was perfomed by Western blot, as described in Materials and Methods. **a** Untreated control cells. **b** TNF-α-treated cells. **c** Compound 1-treated cells. **d** Compound 2-treated cells. **e** TNF-α + compound 1. **f** TNF-α + compound 2. Two Western blot assays were performed.

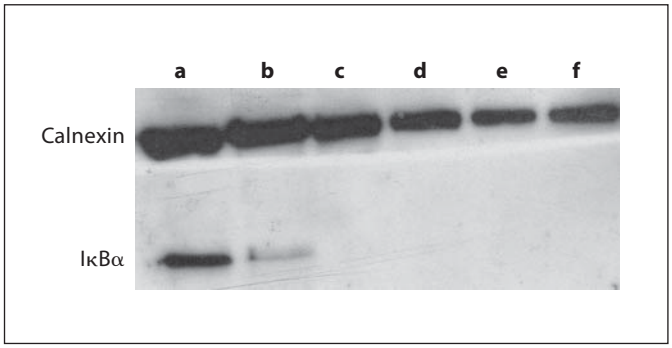


Fig. 7. Effect of triterpene glycosides 1 and 2 pretreatment on TNF-α-induced IκBα degradation in A549 cells. A549 cells were pre-treated either with compound 1 or 2 during 2 h, and then 10 ng/ml TNF-α was added or not during 30 min. The detection of IκBα was perfomed by Western blot, as described in Materials and Methods. **a** Untreated control cells. **b** TNF-α-treated cells. **c** Compound 1-treated cells. **d** Compound 1 (2 h) + TNF-α. **e** Compound 2-treated cells. **f** Compound 2 (2 h) + TNF-α. Two Western blot assays were performed.

origin are being assayed so as to inhibit NF-κB activation and prevent the proliferation of tumoral cells [30, 32–34]. Recently, it has been reported that the monosulfated pentaglycoside frondoside A isolated from *Cucumaria okhotensis* decreased the epidermal growth factor-induced NF-κB-dependent transcriptional activity [23].

Compounds 1 and 2 were not able to suppress NF-κB activation in A549 cells, instead, both promoted NF-κB translocation to the nucleus as well as the degradation of IκBα, the inhibitor protein which retains NF-κB in the cytoplasm, as early as after 30 min and 2 h of treatment (fig. 4, 6). When NF-κB activation was induced by TNF-α, the addition of either compound 1 or 2 did not impede the nuclear translocation of NF-κB (fig. 5, 6). Although NF-κB activation is related to the synthesis of anti-apoptotic molecules, this response would not be enough to impede cellular death as a consequence of the cytotoxic effect of compounds 1 and 2.

These results, together with new mechanisms of action of antitumoral and cytotoxic marine compounds reported in the literature [35] indicate that further pharmacological testing will be required in order to establish a mechanism of action and a clear structure-antiproliferative activity relationship for these marine oligoglycosides.

Acknowledgements

This work was partly supported by the University of Buenos Aires (Grants X046 and X314), the National Research Council of Argentina (CONICET; PIP 5509 and 6033) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT; PICT 14321, 34111 and 38260). C.M., L.A. and M.S.M. are Research Members of CONICET. V.P.C. and C.B. thank ANPCyT and CONICET, respectively, for their fellowships.

References

1 Newman DJ, Cragg GM: Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007;70:461–477.
2 Tian Z, Xu L, Chen S, Zhou L, Yang M, Chen Sh, Xiao P: Cytotoxic activity of schisandrolic and isdoschisandrolic acids involves induction of apoptosis. Chemotherapy 2007; 53:257–262.
3 Mayer AMS, Rodríguez AD, Berlinck GS, Hamann MT: Marine pharmacology in 2003–4: marine compounds with antihelminthic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, an other miscellaneous mechanisms of action. Comp Biochem Physiol C 2007;145:553–581.
4 Park C, Kim GY, Kim WI, Hong SH, Park DI, Kim ND, Bae SJ, Jung JH, Choi YH: Induction of apoptosis by *(Z)*-stellettic acid C, an acetylenic acid from the sponge *Stelletta* sp., is associated with inhibition of telomerase activity in human leukemic U937 cells. Chemotherapy 2007;53:160–168.
5 Maier MS, Murray AP: Secondary metabolites of biological significance from echinoderms; in Fingerman M, Nagabhushanam R (eds): Biomaterials from Aquatic and Terrestrial Organisms. Enfield, Science Publishers, 2006, pp 559–593.
6 Maier MS: Biological activities of sulfated glycosides from echinoderms; in Ur-Rahman A (ed): Studies in Natural Product Chemistry. Amsterdam, Elsevier, 2008, vol 35, pp 311–354.
7 Kalinin VI, Aminin DL, Avilov SA, Silchenko AS, Stonik VA: Triterpene glycosides from sea cucumbers (Holothuroidea, Echinodermata): biological activities and functions; in Ur-Rahman A (ed): Studies in Natural Product Chemistry. Amsterdam, Elsevier, 2008, vol 35, pp 135–196.
8 Zhang ShY, Yi Y, Tang HF: Bioactive triterpene glycosides from the sea cucumber *Holothuria fuscocinerea*. J Nat Prod 2006;69: 1492–1495.
9 Sun P, Liu BS, Yi YH, Li L, Gui M, Tung HF: A new cytotoxic lanostane-type triterpene glycoside from the sea cucumber *Holothuria impatiens*. Chem Biod 2007;4:450–457.
10 Zhang SY, Tang HF, Yi Y: Cytotoxic triterpene glycosides from the sea cucumber *Pseudocolochirus violaceus*. Chem Biod 2006;3: 807–817.
11 Maier MS, Roccatagliata AJ, Kuriss A, Chludil H, Seldes AM, Pujol CA: Two new cytotoxic and virucidal triterpene glycosides from the Antarctic sea cucumber *Staurocucumis liouvillei*. J Nat Prod 2001;64:732–736.
12 Chludil HD, Seldes AM, Maier MS: Antifungal steroidal glycosides from the Patagonian starfish *Anasterias minuta*: structure-activity correlations. J Nat Prod 2002;65:153–157.
13 Chludil HD, Muniain CC, Seldes AM, Maier MS: Cytotoxic and antifungal triterpene glycosides from the Patagonian sea cucumber *Hemoiedema spectabilis*. J Nat Prod 2002;65: 860–865.
14 Chludil HD, Maier MS: Minutosides A and B, antifungal sulfated steroid xylosides from the Patagonian starfish *Anasterias minuta*. J Nat Prod 2005;68:1279–1283.
15 Murray AP, Muniain C, Seldes AM, Maier MS: Patagonicoside A: a novel antifungal disulfated triterpene glycoside from the sea cucumber *Psolus patagonicus*. Tetrahedron 2001;57:9563–9568.
16 Muniain C, Centurión R, Careaga VP, Maier MS: Chemical ecology and bioactivity toxicity of triterpene glycosides from the sea cucumber *Psolus patagonicus* (Dendrochirotida, Psolidae). J Mar Biol Assoc UK 2008; 88:817–823.
17 Denizot F, Lang R: Rapid colorimetric assay for cell growth and survival. J Immunol Methods 1986;89:271–277.
18 Aminin DL, Pinegin BV, Pichugina LV, Zaporozhets TS, Agafonova IG, Boguslavski VM, Silchenko AS, Avilov SA, Stonik VA: Immunomodulatory properties of Cumaside. Int Immunopharmacol 2006;6:1070–1082.
19 Baldwin AS: Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 2001; 107:241–246.

20 Greten FR, Karin M: The IKK/NF-κB activation pathway: a target for prevention and treatment of cancer. Cancer Lett 2004;206: 193–199.
21 Bharti AC, Aggarwal BB: Nuclear factor-kappa B and cancer: its role in prevention and therapy. Biochem Pharmacol 2002;64: 883–888.
22 Roginsky AB, Ding XZ, Kelly DL, Collin P, Talamonti MS, Bell RH, Adrian TE: Transcriptional changes in pancreatic cancer cells treated with Frondoside A. Pancreas 2005;31:465–466.
23 Silchenko AS, Avilov SA, Kalinin VI, Kalinovsky AI, Dmitrenok PS, Fedorov SN, Stepanov VG, Dong Z, Stonik VA: Constituents of the sea cucumber *Cucumaria okhotensis*: structures of okhotosides B_1–B_3 and cytotoxic activities of some glycosides from this species. J Nat Prod 2008;71:351–356.
24 Zou ZR, Yi YH, Wu HM, Wu JH, Liaw, CC, Lee KH: Intercedensides A–C, three new cytotoxic triterpene glycosides from the sea cucumber *Mensamaria intercedens* Lampert. J Nat Prod 2003;66:1055–1060.
25 Zou ZR, Yi YH, Wu HM, Yao X, Du L, Wu JH, Liaw, CC, Lee KH: Intercedensides D-I, cytotoxic triterpene glycosides from the sea cucumber *Mensamaria intercedens* Lampert. J Nat Prod 2005;68:540–546.
26 Yi YH, Xu QZ, Li L, Zhang SL, Wu HM, Ding J, Tong YG, Tan WF, Li MH, Tian F, Wu JH, Liaw CC, Bastow KF, Lee KH: Philinopsides A and B, two new sulfated triterpene glycosides from the sea cucumber *Pentacta quadrangularis*. Helv Chim Acta 2006;89:54–63.
27 Miyamoto T, Togawa K, Higuchi K, Komori T, Sasaki T: Six newly identified biologically active triterpenoid glycoside sulfates from the sea cucumber *Cucumaria echinata*. Liebigs Ann Chem 1990:453–460.
28 Zhang SY, Yi YH, Tang HF: Bioactive triterpene glycosides from the sea cucumber *Holothuria fuscocinerea*. J Nat Prod 2006;69: 1492–1495.
29 Liu HS, Yi YH, Li L, Zhang SY, Han H, Weng YY, Pan MX HF: Arguside A: a new cytotoxic triterpene glycoside from the sea cucumber *Bohadschia argus* Jaeger. Chem Biod 2007;4:2845–2851.
30 Nair AS, Shishodia S, Ahn KS, Kunnumakkara AB, Sethi G, Aggarwal BB: Deguelin: an Akt inhibitor, suppresses IκBα kinase activation leading to suppression of NF-κB-regulated gene expression, potentiation of apoptosis, and inhibition of cellular invasion. J Immunol 2006;177:5612–5622.
31 Sethi G, Ahn KS, Sandur SK, Lin X, Chaturvedi MM, Aggarwal BB: Indirubin enhances tumor necrosis factor-induced apoptosis through modulation of nuclear factor-κB signaling pathway. J Biol Chem 2006;281: 23425–23435.
32 Ma Y-X, Fu H-Z, Li M, Sun W, Xu B, Cui J-R: An anticancer effect of a new saponin component from *Gymnocladus chinensis* Baillon through inactivation of nuclear factor-κB. Anticancer Drugs 2007;18:41–46.
33 Lee J-H, Koo TH, Yoon H, Jung HS, Jin HZ, Lee K, Hong Y-S, Lee JJ: Inhibition of NF-κB activation through targeting IκB kinase by celastrol, a quinine methide triterpenoid. Biochem Pharmacol 2006;72:1311–1321.
34 Ahn KS, Sethi G, Chao T-H, Neuteboom STC, Chaturvedi MM, Palladino MA, Younes A, Aggarwal BB: Salinosporamide A (NPI-0052) potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through down-modulation of NF-κB-regulated gene products. Blood 2007;110:2286–2295.
35 Mayer AMS, Gustafson KR: Marine pharmacology in 2003–4: anti-tumour and cytotoxic compounds. Eur J Cancer 2006;42: 2241–2270.