Cytotoxicity and neurocytotoxicity of new marine anticancer agents evaluated using in vitro assays

Abstract: Purpose: New classes of anticancer drugs, isolated from marine organisms, have been shown to possess cytotoxic activity against multiple tumor types. Aplidine, didemnin B, and isohomohalichondrin B (IHB), among the more promising antitumor candidates, have been evaluated in the present study on a comparative basis in terms of their antiproliferative activity and neurotoxic effects in vitro. Methods: Using a panel of different human, prostatic cancer cell lines (DU 145, PC-3 and LNCaP-FGc) the effects of Aplidine, didemnin B, and IHB on tumor cell proliferation were tested in a colorimetric (XTT) assay and compared with the effects of vincristine, vinorelbine, and Taxol. Under analogous in vitro conditions these drugs were also monitored for neurocytotoxic effects using a PC 12 cell line based model. Results: Didemnin B and – especially – Aplidine were more effective in the inhibition of prostate cancer cell proliferation than vincristine, vinorelbine or Taxol. At these same concentrations, however, Didemnin B and Aplidine were also most potent in the in vitro neurotoxicity assays. IHB was found to exert even more potent antiproliferative activity (at concentration levels between 0.05 and 0.1 pmol/ml). However, neurotoxic effects were also found to be present at these levels. After drug withdrawal, the neurotoxic damage, inflicted by aplidine or IHB appeared to be more long lasting than after vincristine or vinorelbine exposure. Conclusions: These results point to high antiproliferative activity of aplidine and IHB in prostate cancer. At the same time, the data urge some caution in the clinical use of these agents because of potential neurotoxic side-effects. The use of a newly formulated Aplidine may involve a more favorable therapeutic profile.

Key words: Aplidine · Didemnin B · Isohomohalichondrin B (IHB) · Neurocytotoxicity · Cytotoxicity

Introduction

A family of naturally occurring cyclodepsipeptides called didemnins has been isolated from marine tunicates and identified as a class of highly potent anticancer agents [17]. From this family, didemnin B has been tested in phase I and phase II studies for a number of tumors including prostate cancer [29], non-small cell lung cancer [22], myeloma [28], and melanoma [22]. Considerable neuromuscular toxicity has been described as a side-effect of didemnin B in the clinical setting [22, 23]. The mechanism of action of this group of compounds appears to be related to their inhibition of protein synthesis, and to a lesser extent also of DNA and RNA synthesis [13, 25, 27]. More recently, a depsipeptide was isolated from the Mediterranean tunicate, Aplidium albicans, chemically classified as dehydro-didemnin B and later called Aplidine. The preclinical findings for Aplidine point to a potentially high anticancer activity against an array of different, rapidly proliferating tumor types [3, 14, 19, 27]. The possible occurrence of neurotoxic side effects is not yet known.

Another marine organism, the sponge Lissodendoryx spp. was reported to contain the potent isohomohalichondrin B (IHB), with activity against lung cancer and melanoma cell lines [11]. This compound...
transaminase (GTP) binding to tubulin [5]. On the basis of this microtubule interaction, neurotoxicity as a potential side-effect can be anticipated. Therefore, a careful establishment of cytotoxicity and neurocytotoxicity using standardized endpoints under well-controlled conditions seems to be warranted to evaluate the therapeutic potential versus side-effects of both Apilinide and IHB.

We have previously established an in vitro assay for monitoring neurotoxicity of a variety of cytostatic compounds using a pheochromocytoma cell line [6]. This assay has been used to show the differential effects of the various clinically used vinca alkaloids and the possibility of reversibility of neurotoxic effects [9]. The present paper aims at determining in vitro antitumor cytotoxicity using a panel of different human prostate tumor cell lines and comparing antitumor activity with in vitro neurocytotoxicity under identical, standardized conditions in vitro. The experimental drugs are compared with a set of antitumor agents known to display a range of neurotoxic activity in clinical practice.

Materials and methods

Tumor cell lines used and culture conditions

Prostate tumor cell lines (three human derived lines) were used to measure the antitumor cytotoxicity of the drugs. To establish in vitro neurotoxicity a rat pheochromocytoma cell line (PC12) was used. The following three human prostatic cancer cell lines were obtained from the American Type Culture Collection, Rockville, Md., USA:

1. LNCaP-FGC (ATCC# CRL 1740), used: passage 21–80
2. DU-145 (ATCC# HTB 81), used: passage 62–110
3. PC-3 (ATCC# CRL 1435), used: passage 27–85

PC12 rat pheochromocytoma cells were obtained from American Type Culture Collection (ATCC# CRL 1721). Cell culture conditions have been described earlier [7]. In short, all the tumor cells mentioned above were cultured using RPMI 1640 medium (Gibco BRL, Life Technologies, Gaithersburg, Md., USA), supplemented with 10% fetal calf serum (Integro, Zaandam, The Netherlands), 100 U/ml penicillin/streptomycin (Gibco BRL), 1 mM sodium pyruvate (Gibco BRL), and insulin/transferrin/selenium medium supplement (Sigma Chemicals, St. Louis, Mo., USA), at 37 °C in a humidified atmosphere of 5% CO2/95% air.

Cytostatic drugs

- Apilinide (Dehydrodidemmin B), Didemin B, and IHB were kindly provided by PharmaMar through the New Drug Development Office (NDDO) of the EORTC. Apilinide (Dehydrodidemmin B) and Didemin B were dissolved in ethanol absolute (1 mg/ml) and stored in the dark at 4 °C until use.
- Further dilution was performed in phosphate-buffered salt solution (PBS: 0.12 M NaCl/10 mM NaHPO4/3 mM KH2PO4 at pH = 7.4) before addition to the culture medium. Unless otherwise specified, this batch was used as the Apilinide source in the experiments described here under. In two experiments a newly formulated form of Apilinide* [16] was used: 1 mg dissolved in the accompanying 2-ml solution (containing cremophor/ethanol/water for injection). Further dilutions were prepared in PBS immediately before the experiments. IHB was dissolved in ethanol absolute (2 mg/ml) and stored in the dark at 4 °C until use.
- Paclitaxel: Taxol: 2 mg/ml ethanol (Bristol-Myers Squibb, New York, NY, USA)
- Vincristine: Vincristine-sulfate 1 mg/ml (TEVA Pharmaceutical Industries, Netanya, Israel)
- Vinorelbine: Navelbine 50 mg/5 ml (Pierre Fabre Oncologie Laboratories, Boulogne, France)

Drug effect on in vitro prostate tumor cell proliferation: XTT proliferation assay

The effect of the drugs on human prostate tumor cell proliferation was investigated using a commercially available proliferation kit (XTT II, Boehringer Mannheim, Mannheim, Germany). Briefly, the cells were plated in 96-well culture plates (Greiner) at a density of 10 000 cells (LNCaP-FGC); 750 cells (PC-3) or 500 cells (DU-145), respectively, per well in 100-μl RPMI culture medium and allowed to attach for 2 h. Drugs were added to various final concentrations (and control: 0 μM) at various time points and incubated for 72 h after 2 h of culture, 50 μl of XTT reaction solution (sodium 3-[1-phenylaminoacarbonyl]-4,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate and N-methyl dibenzoprazpine methyl sulfate; mixed in proportion 50:1) was added to the wells. The optical density was read at 450 nm wavelength in an ELISA plate reader after 4 h incubation of the plates with XTT in an incubator (37 °C and 5% CO2 + 95% air). All determinations were confirmed using replication in at least three identical experiments. The data shown are for only one experiment, but representative for all replications. Inter-assay variation (CV), for measurement of control culture wells without drug addition, was 4.90% for DU-145 cells, 1.86% for PC-3 cells, and 2.41% for LNCaP-FGC cells, respectively.

In vitro neurotoxicity effects: nerve growth factor-induced neurite outgrowth assay

The nerve growth factor (NGF)-induced neurite outgrowth assay was used as described before [6]. In short, PC12 cells are pretreated for 8 days by adding i-NGF, 2 μg (murine, #G5141, Promega, Madison, Wis., USA) to a final concentration of 50 ng/ml, trypsinized, washed using RPMI 1640 medium and plated in 12-well culture chambers (Costar, Nuclope). Optimal adherence and neurite formation required pre-coating the culture well plastic using a 0.5-μg/ml water solution of polylysine-hydrobromide (P2636, Sigma, St Louis), followed by washing in saline [6]. After 3 days of culture in the presence of NGF (10 ng/ml), the percentage of neurite-forming cells (neurite length >1 × cell body length) is scored in triplicate wells using a phase contrast microscope.

Interference by cytostatic drug with neurite outgrowth was measured by adding the drugs to various final concentrations. To control wells ("0 pmol/ml") PBS solution was added. All incubations were performed in triplicate. After 72 h of culture, in each well 100 cells were randomly chosen and scored for both short (<2 × cell body) and long neurites (>2 × cell body). The results are given as percentage of cells expressing neurites (both short and long) or as percentage of cells expressing long neurites only (>2 × cell body). All determinations were confirmed using replication in at least three identical experiments. The data shown are for only one experiment, but representative for all replications. In order to compare drug effects on established neurites with effects on developing neurites, series of experiments were performed after neurite establishment (48 h culture in presence of NGF followed by 48 h incubation with drugs). The pattern of drug-induced neurite reduction (data not shown) was completely comparable with the effects shown in the present experiments. Inter-assay variation (CV), for determination of neurite-forming cell numbers in control wells without drug addition, was 2.45% for all neurites and 1.89% for long neurites only.