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SHORT COMMUNICATION

Antiproliferative effects of mitraphylline, a pentacyclic oxindole alkaloid of *Uncaria tomentosa* on human glioma and neuroblastoma cell lines

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Abstract

Uncaria tomentosa inner bark extract is a popular plant remedy used in folk medicine to treat tumor and inflammatory processes. In this study, the anti-tumoral effects of its pentacyclic alkaloid mitraphylline were investigated. Furthermore, its growth-inhibitory and cytotoxic effects on glioma GAMG and neuroblastoma SKN-BE(2) cell lines were studied using cyclophosphamide and vincristine as controls. A colter counter was used to determine viable cell numbers, followed by application of the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium], inner salt, colorimetric method to evaluate cell viability in this cytotoxicity assay. Micromolar concentrations of mitraphylline (from 5 to 40 μM) inhibited the growth of both cell lines. It inhibited the growth of the two cell lines studied in a dose-dependent manner. The IC_{50} values were 12.3 μM (30 h) for SKN-BE(2) and 20 μM (48 h) for GAMG, respectively. This action suggests that mitraphylline is a new and promising agent in the treatment of human neuroblastoma and glioma.

Keywords: *Uncaria tomentosa*; Antiproliferative activity; Glioma; Neuroblastoma; Oxindole alkaloid

Introduction

Uncaria tomentosa (Willdenow ex Roemer and Schultes) DC. (Rubiaceae) is a Peruvian thorny liana, which is commonly known as ''uña de gato'' or ''cat's claw''. The aqueous and hydroalcoholic extracts from the bark are used in folk medicine for the treatment of many different health problems, including rheumatism, arthritis, gastrointestinal disorders, weakness, viral infections (including AIDS), skin impurities and as a contraceptive. It is well known that the primary use of the plant is in the treatment of tumor and inflammatory processes (Heitzman et al., 2005).

Cat's claw has been shown to contain many different chemical constituents, including quinovic acid glycosides, sterols, tannins, procyanidins, flavonoids, polyhydroxylated triterpenes and at least 17 different alkaloids (Aquino et al., 1990; De Matta et al., 1976; Ganzera et al., 2001; Kitajima et al., 2002, 2003; Montoro et al., 2004; Muhammad et al., 2001a, b).

There are two botanic chemotypes of this species. Most of the pharmacological activity has been attributed to the one which contains more pentacyclic (rather than tetracyclic) oxindole alkaloids (Philp, 2004). However, the studies have not tested the isolated pentacyclic oxindole alkaloid fractions.

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Numerous investigations on the inmunomodulatory and antiinflammatory properties of different extracts have been reported. There is a very close relationship between the processes. The inhibition of the activation of nuclear factor-κB (NF-κB), which serves as an important regulator of host immune and anti-inflammatory responses (Aguilar et al., 2002), and tumor necrosis factor-α (TNF-α) inhibition tests have demonstrated the ability of *U. tomentosa* extracts to suppress chronic inflammation (Sandoval et al., 2000). The latter is considered the primary mechanism of the anti-inflammatory and inmunomodulatory actions of this species. It has also been shown that cat's claw induces human endothelial cells to release a lymphocyte-proliferation-regulating factor (Wurm et al., 1998) and prolongs lymphocyte survival (Akesson et al., 2003).

On the other hand, no single study has been realized to prove the direct inhibition of brain tumor cell growth by cat's claw. The only study that exists to date is on the effect of different alkaloids from *U. tomentosa* on some leukemia cell lines (Bacher et al., 2006).

The aim of this study was therefore to demonstrate, using an MTS colorimetric method to evaluate cell viability, the antitumoral action of mitraphylline as compared to two well-known citostatic agents, ciclophosphamide and vincristine. We have used two human brain cell lines, neuroblastoma SKN-BE(2) and malignant glioma GAMG.

Materials and methods

Plant extract

The plant material was collected in the Peruvian forest and was provided by Dr. Carlos S. González. The plant extract was produced as follows: 500 g of *U. tomentosa* dried inner bark were treated with ammonium hydroxide and dichloromethane. After filtration, the obtained solution was concentrated in vacuo to yield a residue, which was dissolved in a chlorhydric acid solution (3%). Ammonium hydroxide and dichloromethane were added again. After concentration in vacuo, the purified alkaloid fraction was obtained as a brown residue and the yield was 0.1%.

Isolation and identification of a phytochemical

The dried residue of alkaloid fraction (0.5 g) was subjected to silica gel column chromatography followed by elution with different solvents. The isolated compound was obtained as a solid. EIMS and ^{1}H and ^{13}C NMR experiments were realized for its identification using an AVANCE 500 spectrophotometer. COSY-DQF, NOESY, (^{1}H–^{13}C)–HSQC, (^{1}H–^{13}C)–HMBC and (^{1}H–^{15}N)–HMBC were necessary too. The solvent used for NMR spectra was $CDCl_3$.

Cell cultures

Two cell lines have been used, SKN-BE(2) neuroblastoma (Interlab Cell Line Collection [ICLC] CBA, Genova, Italy) and GAMG glioma (Deutsche Sammlung von Mikroorganismen und Zellkulturen [DMSZ] Braunschweig, Germany). Cell lines were seeded in 75-cm^2 tissue culture flasks (Falcon, Heidelberg, Germany). Both were maintained in RPMI 1640 (neuroblastoma) and Dulbecco's MEM (glioma) supplemented with 10% heat-inactivated fetal bovine serum according to the culture conditions suggested by the DSMZ. The medium was renewed every two days and the cell cultures were incubated at 37 ºC in a humidified atmosphere (95% air and 5% CO_2).

Drug treatments

Mitraphylline, a pentacyclic oxindole alkaloid from the inner bark of *U. tomentosa*, was dissolved in ethanol. In order to determine SKN-BE(2) and GAMG cell proliferation, different concentrations (5, 10, 20, 30 and 40 μM) were evaluated. Cyclophosphamide (Prasfarma, Barcelona, Spain) and vincristine (Ciclum Farma, Madrid, Spain) at the same doses were used as controls.

Proliferation assays

Cell proliferation was evaluated using the tetrazolium compound 3-(4,5- dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS), according to the manufacture's instructions (CellTiter 96 Aqueous One-Solution Cell Proliferation Assay, Promega Corporation, Madison, USA). Both cell lines were cultured for 4–5 days to let them grow in monolayers. Cells were harvested by trypsinization and cell viability. Trypan blue exclusion. Cells were quantified using a colter counter. The experiences were realized in 96-well plates, each well containing 10^4 cells in a total volume of 100 μl. The plates were inoculated with drugs and incubated, respectively, for the first doubling time, 30 h for SKN-BE(2) and 48 h for GAMG. After that, 20 μl of MTS reagent was added to each well. After 90 min, the absorbances of the samples were read at 492 nm in a multiscanner microplate reader (TECAN Spectra classic, Barcelona, Spain). The quantity of product, as measured by optical density, was indirectly proportional to the number of living cells. Each experimental condition was assayed in duplicate and all experiments were performed at least three times (Muñoz et al., 2005).