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The Challenge of the Brain Tumors – Searching for New Therapeutic Opportunities

L. Dyakova[1], D.-C. Culita[2], G. Marinescu[2], L. Patron[2], R. Kalfin[1], R. Alexandrova[3]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 23, Sofia 1113, Bulgaria;*
[2]*Institute of Physical Chemistry "Ilie Murgulescu", Splaiul Independentei 202, Sect. 6, Bucharest 060021, Romania;*
[3]*Institute of Experimental Morphology, Pathology and Antropology with Museum, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 25, Sofia 1113, Bulgaria*

The aim of our study was to evaluate the influence of newly synthesized metal (Zn, Cu, Ni) complexes of ursodeoxycholic acid (UDC) on viability and proliferation of cultured 8 MG BA human glioblastoma cells. The investigations were carried out by thiazolyl blue tetrazolium bromide (MTT) test. The results obtained revealed that applied at concentrations of 10-200 μg/ml for 24 h the compounds examined decreased in a time- and concentration- dependent manner the viability of the treated cells. The copper complex $Cu(UDC)_2 \cdot 2H_2O$ showed relatively the highest cytotoxic/antiproliferative potential. Independently tested, the ursodeoxycholic acid was found to be less active cytotoxic agent as compared to its complexes.

Key words: glioma, cell line, deoxycholic acid, metal complexes, cytotoxicity

Introduction

Gliomas are the most frequent primary neoplasms of the central nervous system (CNS) in adults, consisting of 63% of all primary CNS tumors. The majority of them remain difficult to cure because of the infiltrative growth of cancer cells and their resistance to standard therapy. This is the 3rd leading cause of death from cancer in persons aged 20 to 39 and also the most common solid tumors in children [3, 6, 7]. At the same time there are data revealing that some derivatives of cholic acids decrease the viability and proliferation of cultured human glioblastoma cells [12]. That is why the aim of our study was to evaluate the putative cytotoxic and cytostatic properties of newly synthesized metal complexes of ursodeoxycholic acid in cultured 8 MG BA human glioblastoma cells.

Matherials and Methods

Chemicals and other materials. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco-Invitrogen (UK). Dimethyl sulfoxide (DMSO) and trypsin were obtained from AppliChem (Germany); thiazolyl blue tetrazolium bromide (MTT) were from Sigma-Aldrich Chemie GmbH (Germany). All other chemicals of the highest purity commercially available were purchased from local agents and distributors. All sterile plasticware were from Orange Scientific (Belgium).

Synthesis of metal ursodeoxycholate complexes. The metal complexes containing the anion of ursodeoxycholic acid (UDC) as ligand have been synthesized by the reaction of sodium ursodeoxycholate dissolved in water (at room temperature) with aqueous solution of metal salt $Cu(NO_3)_2 \cdot 3H_2O$; $Ni(NO_3)_2 \cdot 6H_2O$; $Zn(NO_3)_2 \cdot 6H_2O$), under vigorous stirring for one hour. The precipitates were filtered, washed several times with distilled water to eliminate unreacted metal salt and sodium ursodeoxycholate and then desiccated over P_4O_{10}. All of the solid complexes obtained were characterized by elemental chemical analysis and different physico-chemical methods (FTIR, UV-VIS, magnetic measurements, EPR spectroscopy) [5]. The complex compounds have the following formula: $Zn(UDC)_2 \cdot 3H_2O$, $Ni(UDC)_2 \cdot 11H_2O$, and $Cu(UDC)_2 \cdot 2H_2O$. The compounds were initially dissolved in DMSO and then diluted in culture medium. The final concentration of DMSO in stock solutions of the compounds tested (1 μg/ml) was 1-2 %

Experimental part. The permenent human cell line (8 MG BA) established from glioblastoma multiforme was used as an experimental model in our investigations [10]. The cells were grown as monolayer cultures in DMEM medium, supplemented with 5-10% fetal bovine serum, 100 U penicillin and 100 μg/ml streptomycin. The culture was maintained at 37°C in a humidified CO_2 incubator. For routine passages adherent cells were detached using a mixture of 0.5% trypsin (Gibco) and 0.02% ethylendiaminotetraacetic acid (EDTA). The experiments were performed during the exponential phase of cell growth. The effects of the compounds investigated on cell viability and proliferation were evaluated by thiazolyl blue tetrazolium bromide (MTT) test [Mosman]. Relative cell viability, expressed as a percentage of the untreated control (100% viability), was calculated for each concentration and "concentration – response" curves were prepared. All data points represent an average of three independent assays.

Statistical analysis. The received data are presented as mean +/- standard error of the mean. Statistical differences between control and treated groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnett's *post-hoc* test.

Results

The results obtained by MTT test (Fig. 1) revealed that applied at concentrations of 10-200 μg/ml for 24 h the compounds examined decreased in a time- and concentration-dependent manner the viability and proliferation of the treated 8 MG BA glioblastoma cells. The copper complex $Cu(UDC)_2 \cdot 2H_2O$ was found to express relatively the highest cytotoxic/cytostatic propertis. Independently tested, ursodeoxycholic acid was shown to be the less active cytotoxic/cytostatic agent.

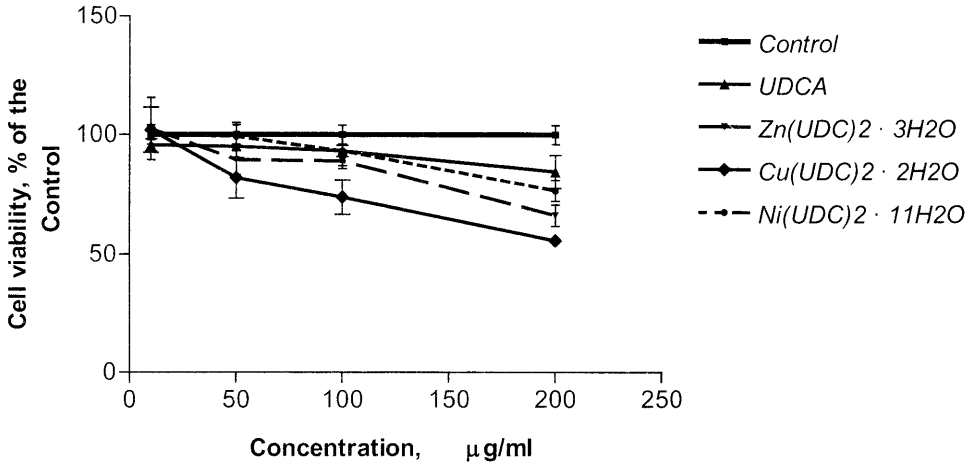

Fig. 1. Influence of ursodeoxycholic acid and its metal complexes on viability and proliferation of cultured human 8 MG BA glioblastoma cells. The compounds are applied at concentrations of 10, 50, 100 and 200 μg/ml for 24 h. The investigations were performed by MTT test.

Discussion

In recent years steroidal structures have become increasingly important in a number of fields such as pharmacology, medicinal chemistry, biomimetic, supramolecular chemistry and also in nanotechnology. There are well known the pharmacological applications of bile acids and their derivatives, including their use in the treatment of liver diseases, in dissolution of cholesterol gallstones, antiviral and antifungal properties, as well as their potential to act as carriers of liver specific drugs and cholesterol level lowering agents. Some of the latest applications in the area of the use of bile acids and their derivatives in cancer therapy are bile acids-*cis*-platin compounds [9] and chenodeoxycholylglycinato derivatives of Pt(II) and Au(III) [4].

A new family complex compounds of transition metals with bile acids and NH_3, named Bamets, have been shown to be promising cytostatic agents [2]. In our investigations the copper complex $Cu(UDC)_2 \cdot 2H_2O$ showed relatively the highest cytotoxic/antiproliferative activity. Copper is known as a trace element which plays a fundamental role in the biochemistry of the human nervous system. On the other hand there are data that some copper containing compounds may possess antineoplastic activity [2, 8, 11]. Something more, since copper is an essential metal for humans it has been suggested that its compounds will be relatively less toxic as compared to those of platinum. In this regard, the compound $Cu(UDC)_2 \cdot 2H_2O$ could be a promising cytostatic agent in glioblastoma treatment.

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