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Cytotoxic and Antiproliferative Activities of a Newly Synthesized Mixed Ligand Cobalt (II) Complex on Tumor Cell Lines

R. Alexandrova, A. Vacheva, I. Todorova, Y. Martinova, E. Nikolova*, E.-M. Mosoarca**, R. Tudose**, O. Costisor***

Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Sofia
**Institute of Morphology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia*
***Institute of Chemistry Timisoara of the Romanian Academy of Sciences*

The aim of this study was to evaluate cytotoxic and antiproliferative activities *in vitro* of a newly synthesized mixed ligand Co(II) complex Co_2(BAMP)py_2Cl_4 (BAMP = N,N′-bis(4-antipyrylmethyl)-piperazine, py = pyridine). The permanent cell lines LSCC-SF(Mc29) (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29) as well as 8 MG-BA (human glioblastoma multiforme) were used in the experiments. The effects of the compound on cell viability and proliferation were studied by neutral red uptake cytotoxicity test, colony-forming assay and autoradiography.

Key words: cobalt, Mannich bases, pyrazolone, cytotoxic/antiproliferative activity, tumor cell lines.

Introduction

Cobalt is one of the most important trace elements in the world of animals and humans. In the form of vitamin B12 (cobalamin) this metal plays a number of crucial roles in many biological functions. Thus, cobalamin is necessary for DNA synthesis, formation of red blood cells, maintenance of the nervous system, growth and development of children. There is evidence to support the role of cobalt in immune processes. A variety of cobalt containing compounds have been proved to possess antineoplastic activity [1, 2]. It was found in our previous investigations that some Cu(I, II), Co(II), Fe(II, III) and Ni(II) complexes with Mannich type ligands - N,N′-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N′-tetra-(antipyryl-1-methyl)-1, 2-diaminoethane (TAMEN), exhibited cytotoxic and antiproliferative effects on several human and animal tumor cell lines [3, 4, 5]. In order to continue the investigations in this field the aim of the study presented here was to evaluate the antitumor activity *in vitro* of a newly synthesized mixed ligand cobalt (II) complex - Co_2(BAMP)py_2Cl_4, containing the above-mentioned Mannich base BAMP as well as pyridine as a coligand.

Material and Methods

The mixed ligand cobalt (II) complex $Co_2(BAMP)py_2Cl_4$ was dissolved in dimethylsulfoxide (DMSO, Serva) and then diluted in culture medium. The final concentration of DMSO in the stock solution of the compound (10 mg/ml) is 10%. The permanent cell lines LSCC-SF(Mc29) (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29) and 8 MG BA (human glioblastoma multiforme), were used in the experiments. Cells were grown as monolayer cultures in a combination (1 : 1, vol. : vol.) of medium H-199 and Minimum Essential Medium (Appli-Chem, Germany), supplemented with 5-10% fetal bovine serum (Cambrex, Belgium), 100 U/ml penicillin and 100 mg/ml streptomycin. The cytotoxic and antiproliferative effects of the compound were studied by neutral red uptake cytotoxicity test, colony-forming assay and autoradiography as it was previously reported [4, 5]. Statistical differences between control and treated groups were assesses using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

Results and Discussion

The data obtained about antitumor activity of $Co_2(BAMP)py_2Cl_4$ *in vitro* are summarized in Table 1. LSCC-SF(Mc29) chicken hepatoma cells were found to be more sensitive to cytotoxic and antiproliferative effects of the compound tested than 8 MG BA human glioblastoma cells. Applied at concentrations ranging from 1 to 200 μg/ml the BAMP ligand did not reduce significantly the viability and proliferation of tumor cells examined.

Independently tested, DMSO (administered at the same concentrations as in the solutions of the compound examined) had no significant cytotoxic effect the viability of the treated cells was > 94% ($P > 0.05$) as compared to the control.

The antitumor [1, 2, 5] and antimicrobial [7, 8] activities of different cobalt compounds have been reported. It was found in our previous investigations that cobalt (II) complexes with N,N′-bis(4-antipyrylmethyl)-piperazine (BAMP) - $Co(BAMP)(NCS)_4$ and $Co_2(BAMP)Cl_4$, possessed more pronounced cytotoxic and antiproliferative properties than the complexes of the same metal with N,N′-tetra–(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) — $Co_2(TAMEN)Cl_4$ and $Co_2(TAMEN)(NCS)_2$ [8]. In this study we report for the first time data about antitumor potential *in vitro* of a newly synthesized mixed ligand Co(II) complex $Co_2(BAMP)py_2Cl_4$, containing not only Mannich base BAMP but also pyridine as a coligand. The results

T a b l e 1. Effect of $Co_2(BAMP)py_2Cl_4$ on viability and proliferation of tumor cells

	LSCC-SF(Mc29)	8 MG BA
Inhibitory concentration 50[a] (IC_{50}, μg/ml) established by Neutral red uptake cytotoxicity assay, determined after 48 h cell treatment with the complex examined	45	100
Concentrations (μg/ml) that inhibit colony-forming ability of tumor cells, determined after 14 day cell cultivation in the presence of of $Co_2(BAMP)py_2Cl_4$	≥ 50	≥ 75
Per cent of ^3H thymidine-labelled cells as compared to the control after 48 h cell treatment with the compound tested	60.23 ± 3.54**	70.53 ± 4.28*

[a] The concentrations producing 50% reduction of neutral red uptake; data represent mean ± SEM (* $P < 0.05$; ** $P < 0.01$)

obtained revealed that this complex expressed time- and concentration-dependent cytotoxic and antiproliferative effects on chicken and human tumor cells used as model systems in the experiments. $Co_2(BAMP)py_2Cl_4$ was found to be much more active as a cytotoxic agent as compared to the previously tested Co(II) complexes with BAMP and TAMEN. Thus, inhibitory concentration 50 (IC_{50}, the concentration producing 50% reduction of neutral red uptake) of $Co_2(BAMP)py_2Cl_4$ for LSCC-SF(Mc29) was calculated to be 45 μg/ml after 48 h treatment whereas IC_{50} for $Co(BAMP)(NCS)_4$, $Co_2(BAMP)Cl_4$ and $Co_2(TAMEN)Cl_4$ were 87, 100 and 98 μg/ml, respectively. Cobalt (II) complexes examined in the study presented here as well as in our previous experiments differ from each other in ligand (BAMP, TAMEN or BAMP + pyridine) and anion (NCS^-, Cl^-). Each of these components (ligands and anions) influences in different way physico-chemical and biological properties of the complexes obtained which could explain the differences in their antitumor effects.

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