ISSN : 0974 - 7435 Volume 11 Issue 12

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(12), 2015 [465-468]

Cytotoxicity action of an oxidovanadium(IV)-DPPZ complex on MG-63 human osteosarcoma cell line

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ABSTRACT

The cytotoxicity activity of the recently reported $[VO(H_2O)_2(SO_4)dppz]\cdot 2H_2O$ complex (dppz = dipyrido[3,2-a:2',3'-c]phenazine) on MG-63 human osteosarcoma cell line was investigated. It was found that it caused a concentration related inhibitory effect in the concentration range between 5 and 25 μM and diminished the cell viability ca. 40% in the range from 25 to 100 μM, without dose/response effects in this range. These biological effects are, in general, similar to those previously reported for the related $[VO(ODA)dppz]\cdot 3H_2O$ (ODA = dianion of oxodiacetic acid) and $[VO(ODA)(phen)]\cdot 1.5H_2O$ (phen = o-phenanthroline) complexes. The importance of the presence of DNA-intercalating ligands in these complexes is emphasized.

KEYWORDS

Oxidovanadium(IV);
Dipyrido[3,2-a:2',3'-c]phenazine (dppz);
Metallointercalators;
Human osteosarcoma cell line.

INTRODUCTION

Vanadium, a group V trace element, belonging to the first transition metal series, has showen to be essential for a number of organisms, for instance, the vanadium containing haloperoxidases found in some marine algae, lichens and fungi, and in the V/Fe nitrogenase of certain *Azotobacter* species. In addition, vanadium is also efficiently concentrated from sea water into the blood cells of tunicates and accumulated in the form of V(III) complexes[1-3]. Although the status of vanadium as an essential trace element in humans remains uncertain[2,3], widespread interest has been directed in recent years to its biological chemistry because its perceived potentiality as a pharmacological agent for the treatment of diabetes and cancer[3-5].

Multiple biological effects of vanadium have been involved in its inhibitory actions on many tumor cells[6]. The fact that bone seems to be the major sink for retained vanadium in the human body[2,4,5], prompted us to investigate the antitumor properties of vanadium complexes on osteosarcoma cell lines (cf. for example[7,8]). Recently, we have prepared a new VO^{2+} complex containing the metallointercalator dppz, of stoichiometry $[VO(ODA)dppz]\cdot 3H_2O$ (ODA

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= di-anion of oxoadiacetic acid, dppz = dipyrido [3,2-a:2',3'-c] phenazine) and investigated its antiproliferative action on MG-63 osteosarcoma cell line[9].

As an extension of this study we have now investigated the cytotoxicity effects of the recently reported and related $[VO(H_2O)_2(SO_4)dppz]\cdot 2H_2O$ complex, which has shown interesting *in vitro* antitrypanosomal activity and cytotoxicity on human promyelocytic leukemia HL-60 cells[10]. Besides, this complex can also be considered as a very good metallointercalator.

Metallointercalators are small complex molecules that contain a planar aromatic heterocycle functionality which can insert and stack between the base pairs of double-helical DNA[11,12]. In general, upon binding to DNA, metal complexes are stabilized through a series of weak interactions such as the π-stacking interactions of aromatic heterocyclic groups between the base pairs (intercalation), hydrogen bonding and van der Waals interactions of functional groups bound along the groove of the DNA helix. In this context, dppz is considered as a very good DNA-intercalator[10-12], which heterocyclic π-system combines the chelating functions of α-diimines or "polypyridines", with the electron transfer/proton transfer capacity of the 1,4-diazines[13].

EXPERIMENTAL

Materials

$VOSO_4\cdot 5H_2O$ and all the employed solvents were from Merck and used as supplied. The dppz ligand was synthesized as described in our previous study[9].

Tissue culture materials were purchased from Corning (Princeton, NJ, USA), Dulbecco´s Modified Eagles Medium (DMEM), TrypLE™ from Gibco (Gaithersburg, MD, USA), and fetal bovine serum (FBS) from Internegocios SA (Argentina). All other chemicals, used in the biological assays, were from Sigma Chemical Co. (ST. Louis, MO). MG-63 cell line was purchased from *ATCC (*CRL1427™).

Synthesis of the complex

The investigated complex, $[VO(H_2O)_2(SO_4)dppz]\cdot 2H_2O$, was prepared using the procedure described by Benítez *et al.*[10], *i. e.*, by reacting an ethanolic solution of dppz with an aqueous solution of $VOSO_4\cdot 5H_2O$. The obtained complex was characterized by elemental chemical analysis (Carlo Erba model EA1108 elemental analyzer) and IR spectroscopy (Bruker EQUINOX 55 FTIR-instrument, KBr pellets).

Cell culture and incubations

MG-63 human osteosarcoma cells (CRL1427™) were grown in DMEM containing 10 % FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ° C in 5% CO_2 atmosphere. Cells were seeded in a 75 cm^2 flask and when 70-80 % of confluence was reached, cells were subcultured using 1mL of TrypLE ™ per 25 cm^2 flask. For experiments, cells were grown in multi-well plates. When cells reached the desired confluence, the monolayers were washed with DMEM and were incubated under different conditions according to the experiments.

MTT Assay

The MTT assay was performed according to Mosmann[14]. Briefly, cells were seeded in a 96-multiwell dish, allowed to attach for 24 h and treated with different concentrations of complexes at 37 °C for 24 h. After that, the medium was changed and the cells were incubated with 0.5 mg/mL MTT under normal culture conditions for 3 h. Cell viability was marked by the conversion of the tetrazolium salt MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide) to a colored formazan by mitochondrial dehydrogenases. Colour development was measured spectrophotometrically in a Microplate Reader (7530, Cambridge Technology Inc., USA) at 570 nm after cell lysis in DMSO (100 μL/well). Cell viability was plotted as the percentage of the control value.

Statistical methods

At least three independent experiments were performed for each experimental condition. Results are expressed as %Basal and represent the mean ± SEM. Statistical differences were analyzed using the ANOVA test.

RESULTS AND DISCUSSION

Structural characteristics of the complex

The analysis of the EPR spectra clearly reveals the presence of a distorted octahedral geometry around the metal center[10], with the dppz ligand acting as bidentate through both N-atoms in an axial-equatorial mode. The oxo group, two H_2O molecules and an O-atom of the sulfate anion (acting as monodentate, C_{3v} symmetry confirmed by the IR spectral characteristics[15]) occupy the remainder coordination positions, as shown in Figure 1.

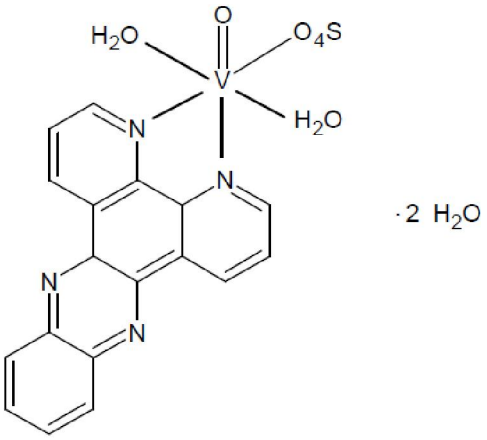

Figure 1 : Schematic structure of the investigated complex

Effects of $[VO(SO_4)(H_2O)_2dppz]\cdot 2H_2O$ on human osteosarcoma cell viability

As mentioned above, and considering the high accumulation of vanadium in bone, it is highly interesting to investigate the effect of new complexes of this element on human osteoblast cells in culture. Therefore, the action on cellular viability of the $[VO(SO_4)(H_2O)_2dppz]\cdot 2H_2O$ complex was determined on MG-63 human osteosarcoma cell line, which is considered as a good model for bone tissue cancer[16].

Figure 2 shows the effects of the $[VOSO_4(H_2O)_2dppz]\cdot 2H_2O$ complex on the mitochondria metabolism of MG-63 osteosarcoma cells. As it can be seen, the complex caused a concentration related inhibition from 5 to 25 μM with statistically significant differences versus basal condition ($p<0.01$). Moreover, the compound impaired the cell viability ca. 40% in the range from 25 to 100 μM ($p<0.01$). Nevertheless, it did not exhibit a dose response effect in this range of concentrations.

Comparing the effects of this complex with those of the recently reported $[VO(ODA)dppz]\cdot 3H_2O$[9], it can be seen that the biological behaviour of both vanadium(IV) complexes on MG-63 cells is very similar in the tested concentrations range (2,5-100

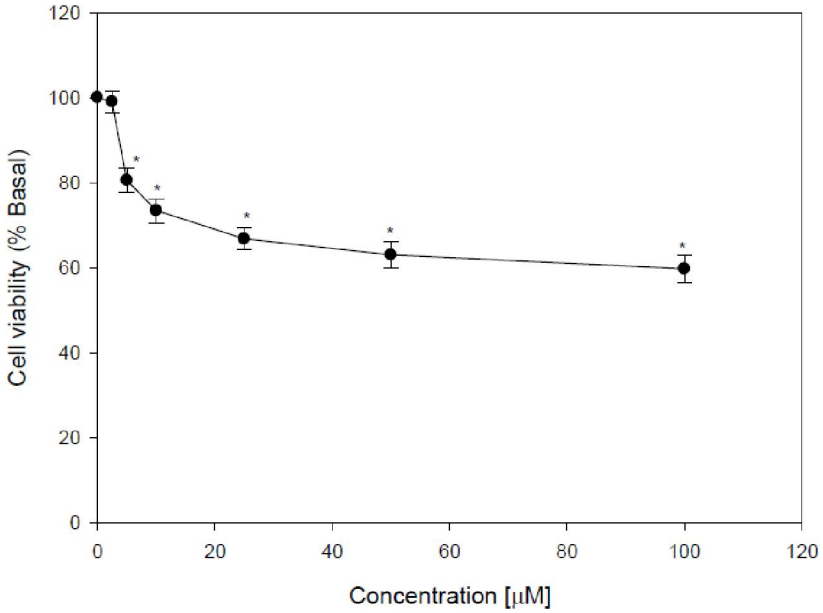

Figure 2 : Evaluation of the mitochondrial succinate dehydrogenase activity by the MTT assay in MG-63 cells in culture. Osteosarcoma cells were incubated with different doses (2,5-100 μM) of the complex for 24 h at 37 ºC. After incubation, cell viability was determined by the MTT assay. Results are expressed as % basal and represent the mean ± SEM, n=18, * significant differences vs. control (p< 0.01)

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μM). These results showed that the change of the tridentate oxodiacetate ligand by the couple of monodentate $SO_4^{2-}/2H_2O$ ligands did not affect the antiproliferative actions of the VO^{2+} complex. On the other hand, $[VOSO_4(H_2O)_2dppz]\cdot 2H_2O$ showed similar biological effects that a previously reported complex of VO^{2+} with oxodiacetate and o-phenanthroline (phen, another recognized DNA-intercallator[11,12]) as ligands, [VO(ODA)(phen)].$1.5H_2O$[8]. As a whole these results support the importance of the presence of intercalating ligands, like phen and dppz, in the anticancer properties of metallic complexes.

ACKNOWLEDGMENTS

This research was supported by the Universidad Nacional de La Plata, the Agencia Nacional de Promoción Científica y Tecnológica-ANPCyT (PICT 2218), and the Consejo Nacional de Investigaciones Científicas y Técnicas-CONICET (PIP 1125 and PIP 5078). The authors are indebted to Dr. J. Zinczuk, Instituto de Química de Rosario (IQUIR/CONICET,UNR), Rosario, Argentina, for the synthesis of dppz. IEL is a fellow from CONICET and SBE is a member of the Research Career from CONICET.

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