In vitro cytotoxic activity of a monolacunary Wells-Dawson nanocluster against cervical carcinoma HeLa cells

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Abstract: The aim of this study was to assess *in vitro* cytotoxic activity of a monolacunary Wells-Dawson nanocluster, α_2-$K_{10}P_2W_{17}O_{61}{\cdot}20H_2O$ (lacunary WD) against cervical carcinoma HeLa cells as a commonly used model system for the evaluation of antitumor properties. After HeLa cells had been exposed to the investigated polyoxotungstate (the concentration range of 0.001 - 1 mM) for 24, 48, and 72 h, relative cell viability (expressed as a percentage of control) was determined. The obtained results showed that lacunary WD affected HeLa cell viability in a concentration- and time-dependent manner. IC_{50} values (in μM), calculated using sigmoidal fitting experimental plots, were as follows: 24.11 ± 9.95, 12.74 ± 0.096, and 11.48 ± 0.12 for 24, 48, and 72 hours treatment, respectively. In comparison with cisplatin, (positive control), IC_{50} values (μM) for 24 hours treatment were similar – 24.11 (lacunary WD) *vs.* 24.49 (cisplatin). However, after 48 and 72 hours IC_{50} obtained for cisplatin were found to be lower – 8.81 and 4.93 μM, respectively. Accordingly, the studied WD polyoxotungstate could not be regarded as a superior anticancer agent in comparison with the standard chemotherapeutic. Nevertheless, this studied nanocluster deserves attention as a promising antitumor therapeutic and as a good platform for the design of next-generation metal-based anticancer agents.

Keywords: antitumor metallodrug, HeLa cells, *in vitro* cytotoxicity, monolacunary Wells-Dawson, polyoxotungstate

1. Introduction

Polyoxotungstates are versatile, metal-based compounds containing tungsten ions in their high oxidation state bridged by oxygen [1]. In recent decades, these negatively charged nanoclusters have been found to demonstrate promising cytotoxic properties against different malignant cells and thus could be studied as a potential platform for the development of next-generation metallodrugs [2].

The objective of this study was to *in vitro* evaluate the antitumor potential of a monolacunary Wells-Dawson polyoxotungstate, α_2-$K_{10}P_2W_{17}O_{61}{\cdot}20H_2O$ (lacunary WD). With this aim, i*n vitro* cytotoxicity was tested using cervical carcinoma HeLa cells as a model system that has been widely used for research purposes.

2. Experimental

Lacunary WD was synthesized according to the literature [3]. A stock aqueous solution of lacunary WD (1 mM) was prepared by vigorous mixing and heating at 30-40 °C, and working solutions were prepared by diluting the stock solution with water up to desired concentrations.

Cervical carcinoma HeLa cells were obtained from the American Tissue Culture Collection (ATCC, Manassas, VA, USA) and cultured in high-glucose DMEM medium (Sigma-Aldrich, Steinheim, Germany) supplemented with 10% fetal bovine serum (Sigma-Aldrich, Steinheim, Germany), and penicillin/streptomycin (Sigma-Aldrich, Steinheim, Germany) in a humidified atmosphere of 5% CO_2 at 37 °C (Heraeus, Hanau, Germany).

Exponentially growing cells, seeded into flat-bottom 96-well plates at a density of 2 × 10^3 cells per well, were treated with the selected concentrations of lacunary WD and cisplatin (*cis*-diamminedichloridoplatinum(II)). The cytotoxicity was determined using a sulforhodamine B (SRB) assay based on the measurement of cellular protein content. The assay was performed according to the method of Skehan *et al*. [4] 24 -72 h after the treatment. Cell monolayers were fixed with 10% trichloroacetic acid (TCA) (Carlo Erba, Milan, Italy) for 1 h at 4 °C and stained with 0.4% SRB (Sigma-Aldrich, Steinheim, Germany). The unbound color was removed, and the plates were washed with 1% acetic acid before air-drying. Protein-bound dye was dissolved in 10 mM Tris base (Sigma-Aldrich, Steinheim, Germany). Absorbance was measured at 550 nm and a reference wavelength of 690 nm using a microplate reader (Wallac, VICTOR2 1420 Multilabel counter, PerkinElmer, Turku, Finland). The obtained results were expressed as relative cell viability, calculated as a percentage of control.

3. Results and discussion

In vitro antitumor action of lacunary WD polyoxotungstate towards cervical carcinoma HeLa cells was evaluated by monitoring relative cell viability expressed as a percentage of control (cell viability obtained in the presence of water). Previously, the investigated cell line was *in vitro* exposed to the selected concentrations of the tested compound (the concentration range of 0.001 - 1 mM) for 24, 48, and 72 h. The obtained

results are presented in Figure 1. In addition, the lacunary WD-induced cytotoxic activity was compared with the antitumor activity of cisplatin against cervical carcinoma HeLa cells under the same conditions, which has been used as a gold standard among metal-based chemotherapeutics.

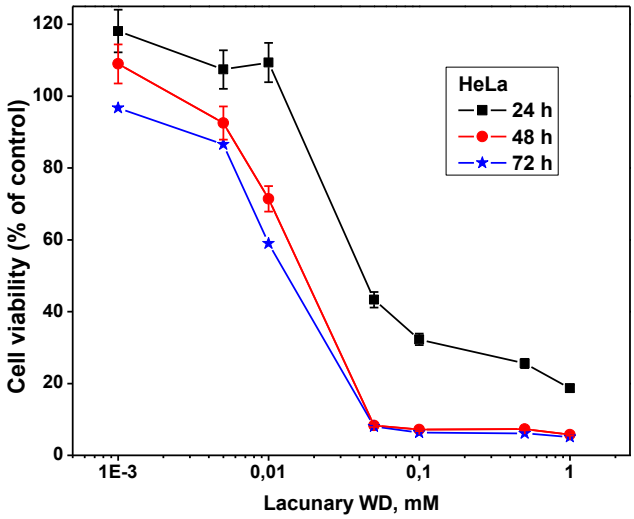

Figure 1. The effect of lacunary WD on the viability of cervical carcinoma HeLa cells. The cells were treated with various lacunary WD concentrations for 24 (square), 48 (circle), and 72 (asterisk) hours. The viability of treated cells was expressed as % of untreated (control) cells (100%). Results are expressed as mean ± S.E.M. from at least 2 independent experiments done in triplicates.

IC_{50} values, used as an indicator of antitumor potency, were calculated using sigmoidal fitting the experimental plots. Calculated IC_{50} values (in μM) were determined as follows: 24.11 ± 9.95, 12.74 ± 0.096, and 11.48 ± 0.12 for 24, 48, and 72 hours treatment, respectively. The obtained results (Figure 1) show that the studied lacunary WD induced HeLa cell cytotoxicity in a concentration - and time-dependent manner. Significant cytotoxic effects were achieved at low micromolar concentrations for all exposure times, whereas concentrations higher than 50 μM resulted in almost complete cell viability reduction after 48 and 72 hours exposure.

In comparison with cisplatin, which was used as a positive control, IC_{50} values (μM) for 24 hours treatment were similar – 24.11 (lacunary WD) *vs.* 24.49 (cisplatin). However, after 48 and 72 hours IC_{50} obtained for cisplatin were found to be lower – 8.81 and 4.93 μM, respectively. This indicates that the studied WD polyoxometalate could not be considered a superior anticancer agent in comparison with the standard chemotherapeutic.

4. Conclusions

The results of this *in vitro* study on the potential antitumor activity of lacunary WD polyoxometalate demonstrated that the synthesized polyoxotungstate induces

significant cytotoxic effects on cervical carcinoma HeLa cells at low micromolar concentrations, which are comparable with the cytotoxic properties of cisplatin, the gold standard chemotherapeutic. Thus, this studied nanocluster deserves attention as a potential antitumor therapeutic and as a good base for the design of novel metal-based anticancer agents.

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